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Vol. 56, No. 1

Cancer Chemotherapy Reports

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Use of funds for printing this publication approved by the Director of the Bureau of the Budget, September 21, 1967

Intestinal Absorption of Phenesterin (NSC-104469), An Alkylating Cholesterol Ester[1, 2, 3, 4]

Elaine B. Feldman, Henry Paul, and Robert Cheron[5,6]

SUMMARY

The intestinal absorption of phenesterin, an alkylating cholesterol ester, radiolabeled with ^{14}C in the bis-chlorethyl moiety and ^{3}H in cholesterol, was studied. This unique cholesterol ester was not hydrolyzed before uptake by hamster small intestinal rings. When fed by gavage to hamsters, phenesterin was recovered intact in the lumen of the stomach, small intestine, and large intestine, confirmed by thin-layer chromatographic separation from cholesterol and cholesterol esterified with fatty acid. In the small intestinal wall, phenesterin was hydrolyzed 4–6 hours after feeding, and ^{3}H was recovered in free cholesterol and in cholesterol esterified with fatty acid. The rate of phenesterin hydrolysis was more rapid in the intestinal wall of thoracic duct-cannulated rats. ^{3}H radioactivity reached a peak 5 hours after the oral dose in thoracic duct lymph of rats. For the first 3 hours phenesterin was recovered intact in the lymph; after 7 hours most ^{3}H radioactivity in the lymph was in esterified cholesterol. ^{14}C radioactivity was appreciable in portal plasma 2–4 hours after feeding rats. Overall absorption of phenesterin in rodents was estimated to be about one tenth that of cholesterol.

[Cancer Chemother Rep 56:1–7, 1972]

Phenesterin, an ester of cholesterol with *p*-[bis (2-chloroethyl) amino] phenylacetic acid (NSC-71964), is a complex alkylating metabolite synthesized by Shkodinskaya in 1962 (fig 1) (1). It is a selective chemotherapeutic agent, with a cytotoxic group esterified to a primary carrier, bound to a terminal lipophilic compound. Its antitumor effect in animals is at least equivalent to that of cyclophosphamide and about 3 times that of chlorambucil, with less toxicity (2).[7] The drug is more effective in rats at doses of 10 mg/kg/day given orally than by subcutaneous or intraperitoneal administration.[8]

Hydrolysis of cholesterol esters is apparently an absolute requirement for their intestinal absorption in the rat (3,4). The enhanced anti-

[1] Received Mar 11, 1971; revised May 27, 1971; accepted June 14, 1971.

[2] Presented in part at the 14th International Conference on the Biochemistry of Lipids, Lund, Sweden, June 1970.

[3] Supported in part by grants HE-10742 from the National Heart Institute and CA-3358 from the National Cancer Institute (NCI).

[4] Phenesterin: cholesterol, [*p*-bis (2-chloroethyl) amino] phenyl]-, acetate.

[5] Department of Medicine, State University of New York, Downstate Medical Center, Brooklyn, and The Will Rogers Summer Research Institute, Saranac Lake, NY.

[6] Charles Cheng and Leonard Mestrandrea provided excellent technical assistance. Drs. Roland Girardet and Julia Quagliata performed some of the thoracic duct cannulations.

[7] Cyclophosphamide: NSC-26271; 2*H*-1,3,2-oxazaphosphorine, 2-[bis (2-chloroethyl) amino] tetrahydro-, 2-oxide, monohydrate.

Chlorambucil: NSC-3088; butyric acid, 4-[p-[bis (2-chloroethyl) amino] phenyl]-.

[8] Vollmer, E. P., Taylor, D. J., Masnyk, I. J., et al. Clinical brochure: phenesterin (NSC-104469), 1969. Prepared by the NCI, Bethesda, Md.

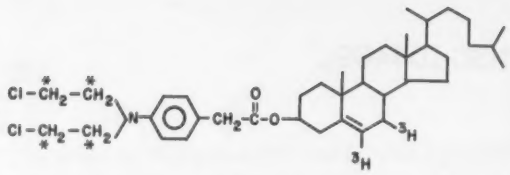

FIGURE 1.—Chemical structure of phenesterin. Molecular formula: $NO_2Cl_2C_{30}H_{50}$.

tumor potency and decreased toxicity of phenesterin when given by mouth suggested that the compound might be transported intact through the gut and be split into alkylating and carrier portions in the peripheral tissues. The mechanisms of absorption of this unusual cholesterol ester were therefore investigated. It was demonstrated that phenesterin was not hydrolyzed initially in chyme, was taken up intact by the small intestine, and was partly split in the intestinal wall. Overall, about 25% of the compound was recovered unhydrolyzed in the thoracic duct lymph.

MATERIALS AND METHODS

Radiolabeled phenesterin, ^{14}C labeled in the ethyl moieties of the "mustard" and ^{3}H labeled in cholesterol, was synthesized and provided by Dr. M. Wall, Research Triangle Institute (5). Specific activities of three batches were 1.10, 1.62, and 1.77 μc/mg for the ^{14}C-labeled drug, and 4.86, 5.51, and 5.58 μc/mg for the ^{3}H-labeled drug. Unlabeled phenesterin was provided by Dr. E. Vollmer, Endocrine Evaluation Branch, NCI.

The compound was stable as a solid when stored in the dark at 4 C. It dissolved readily in benzene or dimethyl sulfoxide. Chemical purity and radiopurity were assessed at greater than 98% by thin-layer chromatography on silica gel G using developing solvent hexane, diethyl ether, and acetic acid (79:20:1, v/v).

In Vitro Studies: Hamsters

Jejunal rings of hamsters were incubated for 30 minutes (6) with 1 ml mixed micellar solutions of 5.4 mM monoolein[9] and 6.0 mM sodium taurodeoxycholate[10] in Krebs-Ringer phosphate buffer (pH 6.3) containing radiolabeled and unlabeled phenesterin. Drug concentrations of 0.05–0.5 mM were achieved by dissolving phenesterin in 0.01 ml dimethyl sulfoxide per milliliter of solution.

Lipids were extracted from solutions and homogenized tissues using chloroform-methanol (2:1, v/v). The organic phase containing extracted phenesterin and other lipids was separated, dehydrated, and dried. Lipids were resuspended in chloroform. Portions of lipid extracts were applied to thin-layer silicic acid plates. Lipids were separated by ascending thin-layer chromatography using developing solvent hexane, diethyl ether, and acetic acid (79:20:1, v/v). Compounds were sprayed with iodine solution and identified by comparison

[9] Distillation Products Industries, Rochester, NY.

[10] Maybridge Chemical Co., Tintagel, England.

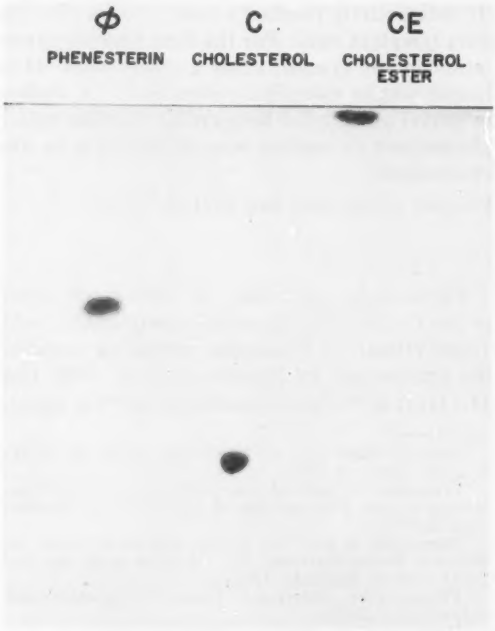

FIGURE 2.—Separation of phenesterin from cholesterol and cholesterol esters by thin-layer chromatography on silica gel G. Plates were developed in hexane, diethyl ether, and acetic acid (79:20:1, v/v). Spots were visualized by spraying with sulfuric acid and charred for photographic purposes.

with pure reference standards. Phenesterin was well separated from free and esterified cholesterol (fig 2). These spots were scraped into scintillation vials. Radioactivity was measured in a liquid scintillation spectrometer (Beckman LS 100 or 250), using scintillation counting fluid of naphthalene (100 g) and 2,5-diphenyloxazole (10 g) per liter of dioxane. Quenching was corrected by use of external standards. Radioactivity was expressed as dpm/ml for each isotope, and the ratio of ^{3}H:^{14}C was calculated. Radioactivity of portions of lipid extracts was also determined directly.

In Vivo Studies: Anesthetized Hamster

A cannulated jejunal segment in an anesthetized hamster was recirculated in vivo for 2 hours with a phenesterin solution pumped at 1 ml/minute. Lipids were extracted from the perfusing solutions at the beginning and end of the experiment, and from the homogenized jejunum, and processed as described in the previous paragraph.

One-Dose Administration: Hamsters

A dose of 2.5 mg of phenesterin, including 0.5–1.69 mg of radiolabeled drug, was fed by stomach tube to 16 male hamsters (weight, 114 g) under light ether anesthesia. The drug was given in 0.4–0.6 ml of dimethyl sulfoxide [No. of animals (n) = 9], mixed micellar solution (n = 2) or triolein emulsion (n = 2), or sesame oil (n = 3), and washed down with additional 0.2–0.4 ml of diluent. Animals were killed by decapitation 2, 4, and 6 hours after feeding. The contents of the stomach, small intestine, and large intestine were aspirated and rinsed with Krebs-Ringer phosphate buffer containing 6 mM sodium taurodeoxycholate. The tissues of the gastrointestinal tract, the tissues of the liver, and the feces were homogenized and extracted in chloroform-methanol. Gastrointestinal contents were extracted with equal volumes of ethanol, diethyl ether, and heptane. Radioactivity of the rinsed contents of the gastrointestinal tract was determined directly by adding a portion to scintillation fluid.

One-Dose Administration: Rats

Thoracic duct cannulation (8) was performed in five rats, 24 hours before feeding phenesterin given in dimethylsulfoxide (n = 1), mixed emulsion (n = 1), or triolein (n = 3). Animals were kept in restraining cages and had free access to 5% glucose in isotonic saline for drinking; food was withheld. Phenesterin was fed by gavage. Fluids were then withheld for 3 hours after which the rats had free access to water. Lymph was collected hourly in heparinized tubes kept on ice. Radioactivity of lymph was determined directly by adding 0.2 ml portions to scintillation fluid. The remaining lymph was extracted with chloroform-methanol and processed as other lipid extracts. Animals were killed 6 and 24 hours after feeding phenesterin.

Portal blood was aspirated from four other rats under ether anesthesia (9) 2, 3, and 4 hours after feeding phenesterin in triolein. Rats were given 75 units of heparin by femoral vein cannula 10 minutes before laparotomy so that blood could be collected over several minutes. Radioactivity of separated plasma was measured by adding 1 ml to scintillation fluid.

RESULTS

In Vitro Studies: Hamsters

Jejunal rings took up 0.155 μmol/g of radiolabeled phenesterin in 30 minutes from incubation media containing 0.2 mM phenesterin (n = 6). Tissue uptake was in linear proportion to concentration in the medium. Mean phenesterin uptakes for 0.05, 0.1, 0.25, and 0.5 mM solutions were 0.052, 0.102, 0.171, and 0.446 μmol/g respectively. Recovery of radioactivity from incubation solutions and tissues was 93% of the amount added. In the jejunum, radioactive phenesterin, isolated by thin-layer chromatography, accounted for 92% of the radioactivity of the lipid extract.

In Vivo Studies: Anesthetized Hamsters

Phenesterin did not break down in perfusion fluid recycled through isolated cannulated jejunum for 2 hours in an anesthetized hamster. Radioactivity recovered in the jejunal wall at

the end of the experiment was localized entirely to the position of phenesterin on the thin-layer chromatogram. Phenesterin radioactivity disappeared from the perfusing solution at a rate comparable to that observed in similar experiments using ^{14}C cholesterol (45% vs 33%). Radioactivity recovered in the jejunal wall after phenesterin perfusion was one twelfth the amount observed with cholesterol, free and esterified (0.03 vs 0.46 μmol/g jejunum).

Feeding: Hamsters

Phenesterin left the stomach rapidly and appeared in the small intestinal content 1 hour after gastric intubation (fig 3). Peak recovery in the small intestinal wall occurred 2–4 hours after feeding. By 4 hours, phenesterin appeared

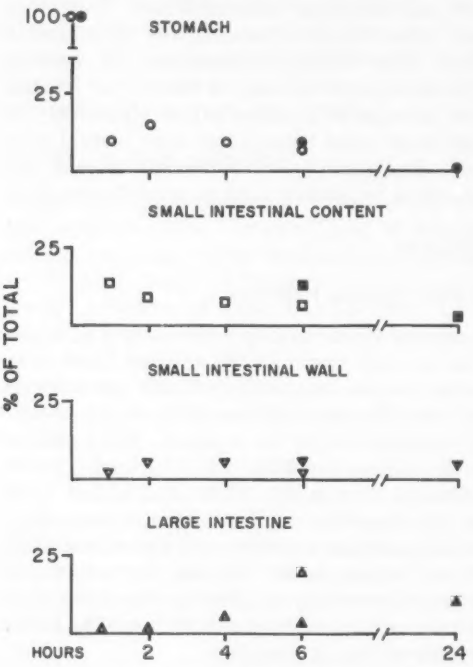

FIGURE 3.—Appearance of radioactivity along the gastrointestinal tract at varying time intervals after feeding ^{3}H- and ^{14}C-radiolabeled phenesterin to 16 hamsters and 9 rats. Values on ordinate represent mean percent of administered dose of ^{3}H. Open symbols represent mean recoveries in hamsters at 1 hour (n = 1), 2 and 4 hours (n = 3), and 6 hours (n = 9). Closed symbols represent data in rats at 6 hours (n = 1) and 24 hours (n = 4).

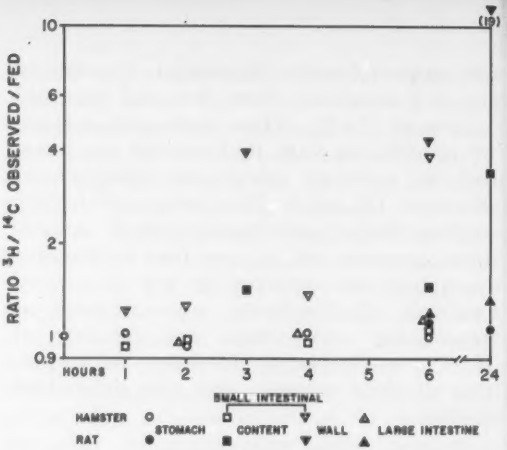

FIGURE 4.—Ratio of ^{3}H:^{14}C radioactivity in gastrointestinal contents and tissues at various time intervals after feeding radiolabeled phenesterin expressed as a function of the ^{3}H:^{14}C ratio of the fed compound. See figure 3 for definition of symbols. Data in rats are also included at 3 hours (n = 2).

in the large intestinal lumen. By 6 hours, most of the compound recovered was in the large intestine.

The ^{3}H:^{14}C ratio of phenesterin in the stomach and the small intestinal content was unchanged at 1, 2, 4, and 6 hours from that of fed phenesterin (fig 4). This would imply that the ^{3}H- and ^{14}C-radiolabeled moieties had not separated and remained either as intact phenesterin or some similar product including both isotope labels which had the same mobility by thin-layer chromatography. In the large intestine, isotope ratios were unchanged at 4 hours and were increased 15% at 6 hours. After thin-layer chromatography, 3% of the ^{3}H radioactivity of extracted gastric contents and 10% of small intestinal chyme were located in the cholesterol "spot" at 6 hours (fig 5).

In extracts of the small intestinal wall, isotopic ratios of ^{3}H:^{14}C were increased about 30% by 4 hours (fig 4), and then increased sharply 4-fold at 6 hours. After extracts of the small intestinal wall were chromatographed at 1 and 2 hours, one fourth of the radioactivity was recovered in free (19%) or esterified (5%) cholesterol. At 6 hours, almost half of the radioactivity was recovered as free (38%) or esterified (8%) cholesterol (fig 5).

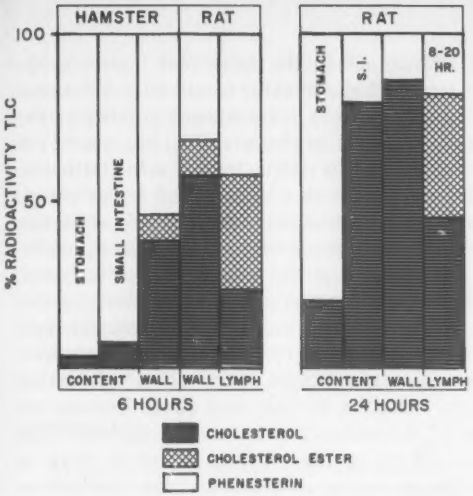

FIGURE 5.—Distribution of ^{3}H radioactivity recovered from plates after thin-layer chromatographic separation of phenesterin (clear), cholesterol (solid), and cholesterol esters (x-hatch) in lipid extracts of stomach, small intestinal content and wall, and lymph of 9 hamsters and 1 rat 6 hours after feeding radiolabeled phenesterin and in 4 rats at 24 hours. Developing solvent is described in figure 2.

Feeding: Rats

Transit time through the small and large intestine was slower in the rat than in the hamster. The transit of phenesterin was similar to cholesterol (10,11).

In the small intestinal chyme of rats, ^{3}H:^{14}C ratios were increased 40% at 3–6 hours and more than 300% at 24 hours. At 6 and 24 hours, there was a 15%–25% increase in ^{3}H:^{14}C in the large intestine. At 24 hours 20% of ^{3}H radioactivity in the gastric contents (fig 5) and 80% of radioactivity in the rat chyme after thin-layer chromatography were recovered as cholesterol. At 24 hours 15% of stool radioactivity was recovered from the cholesterol "spot."

Isotope ratios in extracts of small intestinal wall were increased 4- to 5-fold at 3–6 hours, and were increased 19-fold at 24 hours (fig 4). In the rat small intestinal wall at 6 hours, two thirds of the radioactivity was recovered as free (58%) and esterified (10%) cholesterol (fig 5). At 24 hours breakdown was 86%, recovered entirely as free cholesterol.

Measurable radioactivity appeared in lymph

TABLE 1.—Recovery of ^{3}H radioactivity, isolated by thin-layer chromatography, in lymph after feeding radiolabeled phenesterin*

Hours after feeding	Percent recovered†		
	Phenesterin	Cholesterol	Cholesterol esters
3	74	13	13
4	50	29	25
5	39	14	50
6	31	23	34
7	25	24	52
8	25	30	46
8–20	28	45	37
Total recovered	25	23	26

*One mg radiolabeled phenesterin was fed by gavage to 5 rats with lymph fistulas. One animal was killed at 6 hours and 4 at 24 hours.

†Percent = mean value

$$\frac{\text{dpm in compound (phenesterin, cholesterol, esters)} \times 100}{\Sigma \text{ dpm recovered from chromatography plate}}.$$

Lipid extracts of lymph samples were chromatographed.

3 hours after intragastric tube feeding (fig 6). Peak radioactivity was reached at 5 hours, and declined over the 20-hour collection period which was stopped when radioactivity in lymph was negligible. Half of the total radioactivity recovered in lymph was collected by 6 hours, two thirds by 8 hours. Isotopic ratios of ^{3}H:^{14}C

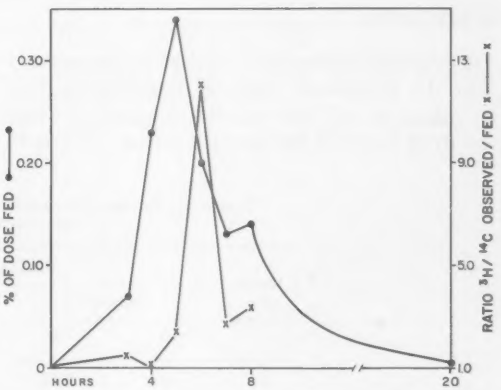

FIGURE 6.—Appearance of radioactivity in lymph at varying time intervals after feeding radiolabeled phenesterin to 5 rats with lymph fistulas. The ^{3}H:^{14}C ratio of lymph extracts is expressed as a function of the ^{3}H:^{14}C fed.

peaked sharply at 6 hours to a value 12 times the fed value. Radioactivity recovered as phenesterin in lymph declined from 3 hours on, and radioactivity in cholesterol esters increased (table 1, fig 5). At 3–4 hours three fourths of ^{3}H radioactivity was recovered from the phenesterin "spot"; from 7 hours on, phenesterin radioactivity accounted for only one fourth of the radioactivity plated. At 5–8 hours, half of ^{3}H radioactivity was recovered from the cholesterol ester "spot." Cholesterol ester radioactivity ranged from 43% to 78% of total cholesterol radioactivity, with a peak at 5 hours and an average of 53% over the entire period of lymph collection.

Radioactivity, by direct measurement of portal plasma, increased over the 2–4 hour period of collections (table 2). The isotopic ratio of ^{3}H:^{14}C was decreased at 2 hours to 3% of the fed value and at 4 hours to 24% of the fed value. Radioactivity recovered from systemic venous blood plasma and liver was about 70% in the form of free cholesterol (52%) and esterified cholesterol (18%).

Total recovery of radioactivity from gastrointestinal contents and tissues of fed hamsters or rats averaged about 40% for phenesterin in contrast to 84% when 2 μc of ^{3}H-labeled cholesterol was fed (1.7 mg) in comparable experiments.

DISCUSSION

The experimental data support the hypothesis that the antitumor effect of phenesterin may be enhanced and the toxicity diminished when the drug is given by mouth because: (*a*) it is not hydrolyzed in the intestinal lumen as an ordinary cholesterol ester would be but is taken up intact; and (*b*) a significant portion of the drug is not split in the intestinal mucosa but is delivered intact via the lymph into the blood.

The compound may be delivered to peripheral tissues intact and changed at the site of action. The rate of delivery of the drug, or the split-off alkylating portion, from intestine to lymph and blood is slow and sustained presumably due to the slow rate of transport of cholesterol from intestine to lymph (9,10). Thus, phenesterin may have advantages over other alkylating agents effective by the oral route because its rate of decomposition in vivo is slower (12). The oral route may deliver effective drug to the tissues more consistently than parenteral administration. Certainly phenesterin is less toxic than its mustard derivative phenester (Taylor, D. J., personal communication). Because of its lipid solubility the lipophilic carrier cholesterol may transport the alkylating agent more effectively to many cells, particularly to areas like the nervous system, rich in tissue lipids, or to adipose tissue.

Phenesterin apparently was not hydrolyzed by pancreatic cholesterol ester hydrolase, since breakdown in intestinal chyme occurred only late and presumably was non-enzymatic. Partial breakdown in the small intestinal wall during absorption probably occurred by action of intestinal cholesterol esterase. The intact phenesterin which entered lymph may have been incorporated into chylomicrons similar to fatty acid esters of cholesterol. The cholesterol, split from the chlorphenacyl moiety, underwent the usual fate of absorbed sterol:

TABLE 2.—Recovery of radioactivity in portal plasma after feeding radiolabeled phenesterin*

Hours after feeding	Radioactivity				^{3}H:^{14}C recovery/ ^{3}H:^{14}C fed
	Dpm/ml		Percent of dose		
	^{3}H	^{14}C	^{3}H	^{14}C	
2	497	4385	0.025	0.7	0.03
3	1444	4775	0.33	0.95	0.10
4	15399	21651	0.8	3.0	0.24

*Radiolabeled phenesterin was fed by gavage to 4 rats. Portal blood was drawn at 2, 3 (n=2), and 4 hours from heparinized animals under ether anesthesia. Plasma radioactivity was counted directly.

esterification with fatty acid with prompt release into lymph (11). Some free sterol entered lymph and some was "secreted" into the gut lumen (12). The mustard moiety released evidently entered the portal system; the rate of release and fate of the mustard moiety is unknown. Presumably, phenesterin is susceptible to hydrolysis by tissue cholesterol esterases; such enzymes are active in a variety of tissues including liver, adipose tissue, and adrenals (14).

Absorption of phenesterin was not studied quantitatively in vivo. Nonetheless, the absorbed compound was recovered to a limited extent, estimated at about 10% of equivalent amounts of cholesterol (10, 15), and similar to recovery of absorbed cholesterol decylether or plant sterols (16). The degree of absorption is sufficient for the drug to be an effective chemotherapeutic agent. Micellar solubilization of phenesterin in bile salt-monoolein was poor compared to cholesterol, and may correlate with its lesser absorption. More of the mustard moiety may be absorbed by the portal system, but quantitation is difficult.

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Tumor-Inhibitory Effect of a Streptococcal Preparation (NSC-B116209) [1]

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SUMMARY

The cell suspension of a low virulent strain, Su, of *Streptococcus hemolyticus* (Group A) treated with penicillin G (NSC-402815), OK-431, and its lyophilized preparation, OK-432 (NSC-B116209), were found to be effective against various kinds of rat ascites tumors in vivo. The preparation was also effective against ascites and solid forms of Ehrlich carcinoma in mice. Pretreatment of rats with the preparation before inoculation of rat ascites hepatoma caused a significant prolongation of lifespan of the tumor-bearing animals. In vitro contact of Yoshida sarcoma or rat ascites hepatoma cells with the preparation showed direct cytocidal action. From these results, this preparation seemed to have both direct and host-mediated antitumor activity, which might be responsible for the antitumor effect of this preparation in vivo. In acute toxicity tests in animals, pyrexia and leukocytosis were observed. Agglutinin against *Streptococcus hemolyticus* (Su strain) was slightly increased by repeated injections. No changes in the bone marrow were noted histologically in a long-term toxicity test.

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Since Busch (1) published his noteworthy report in 1868 that a human malignant tumor regressed when the patient had erysipelas, many investigators (2–9) have tried to make use of hemolytic streptococci for finding a new approach to the treatment of human cancers. The effect of streptococci on human tumors, however, has not been clearly proved.

In 1940, Okamoto (10) reported his investigations into streptolysin S production by hemolytic streptococci. Koshimura found in 1955 (11) and 1958 (12) that degradation and loss of transplantability of Ehrlich carcinoma cells occurred together with the production of streptolysin S when a suspended mixture of tumor cells and living cells of *Streptococcus hemolyticus* was incubated at 37 C for 90 minutes. Together, they studied further the correlation between antitumor effect and streptolysin S production of the streptococcal cell suspension. The antitumor effect of the suspension was lost by sonication, heating at 100 C, or formaldehyde treatment (13). In 1966, they reported (13,14) that the cell suspension of a low virulent strain, Su, of *Streptococcus hemolyticus* in Bernheimer's basal medium (BBM) (15), attenuated by the addition of penicillin G (NSC-402815) and heated at 45 C, exhibited a strong inhibitory effect on the growth of Ehrlich carcinoma transplanted intraperitoneally into mice. The antitumor effect of this preparation was enhanced about 20-fold from that of the original streptococcal cell suspension. It was also proved that the attenuated preparation was completely devoid of streptolysin S-producing ability and showed no sign of systemic infection in animals when injected through various routes. However, when a physiologic

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saline suspension of streptococcal cells was heated at 45 C in the presence or absence of penicillin G, there was little antitumor effect. Its inhibitory effect on another transplantable tumor, Sarcoma 180, has been confirmed by the same authors.[5]

This paper presents the results of extended experiments studying the inhibitory effect of the lyophilized preparation of *Streptococcus hemolyticus* (Su strain) on transplantable tumors and its toxicity in laboratory animals.

MATERIALS AND METHODS

A low virulent strain, Su, of *Streptococcus hemolyticus* (Group A), isolated by Okamoto and his co-workers, was cultivated in meat-infusion broth at 37 C for about 20 hours. The culture was then inoculated into 3% yeast extract medium and cultivated at 37 C for 20 hours. The cells were collected by centrifugation and washed twice with a chilled physiologic saline solution. The cells were then suspended in 5 volumes of BBM,[6] and 1 volume of physiologic saline containing penicillin G (1.6×10^5 units/ml) was added. The final cell concentration was made with 5 mg of dried cells per milliliter. After being cultivated at 37 C for 20 minutes, the cell suspension was further heated at 45 C for 30 minutes. This preparation is designated as OK-431.[7] It was lyophilized after the addition of an equivalent volume of aqueous 1% DL-methionine solution containing 1.08×10^5 units/ml of penicillin G. This lyophilized preparation is designated as OK-432 (NSC-B116209). OK-432 is a white hygroscopic powder, which is suspended in physiologic saline before use. The cells contained in OK-432 did not grow in the usual culture medium after the removal of penicillin G. The antitumor activity of OK-431 is relatively unstable and is lost rapidly when kept at room temperature or diminishes after 3 weeks when kept in a refrigerator. On the other hand, antitumor activity of OK-432 was not lost when kept at room temperature for a fairly long period, and as long as it was kept in a freezer (–20 C) the activity was maintained for at least 2 years.

To express the cell content in OK-431 or OK-432, a Klinische Einheit (KE) system was adopted. One KE corresponds to the amount of preparation containing 0.1 mg dried cells.

Animal Studies

For the study of toxicity, the following animals were used: 6-week-old ddY mice, weighing 18.5–21.5 g; Wistar-Imamichi rats, weighing 190–235 g; Hartley guinea pigs, weighing 600–800 g; Japanese albino rabbits, weighing 2.8–3.4 kg; cats, weighing 2.5–3.1 kg; beagle dogs, weighing 8.0–12.0 kg; and Japanese monkeys (*Macaca fuscata Yaku*), weighing 3.5–5.0 kg. For tests of antitumor effect, Donryu rats, weighing 100–150 g, were used. For leukemia L1210, CDF$_1$ (BALB/c female × DBA/2 male)F$_1$ mice weighing 20–27 g, and for Ehrlich carcinoma, ddY mice weighing 20–25 g, were used.

Toxicity Tests

The LD50 was calculated by the Litchfield-Wilcoxon method using the criterion of death within 7 days after single administration of OK-432 in suspension.

For subacute toxicity tests, animals were given the preparation in various dosages daily for 4 weeks and their symptoms were observed. The surviving animals were then killed and examined histopathologically.

In Vitro Antitumor Tests

The in vitro antitumor effect was examined according to the method described by Sakurai in 1964 (16).

For the in vitro test of antitumor activity of OK-431 and OK-432, Yoshida sarcoma or AH-13 ascites cells were harvested from the peritoneal cavity of rats bearing 4-day-old tumors. The cells were suspended in the following medium: Hanks' saline solution (containing 0.4% lactalbumin hydrolysate), 79 vol; potassium penicillin G (100 units/ml), 1 vol; horse serum (inactivated at 56 C for 30 min), 20 vol. In this medium, the final cell concentration was adjusted to about 5×10^4 cells/ml and the test

[5] Koshimura, S., Shoin, S., and Okamoto, H. Work in preparation.

[6] BBM was composed of 675 mg maltose, 6 ml 20% KH_2PO_4 (adjusted to pH 6.9–7.0 with NaOH), 12 ml 2% $MgSO_4 \cdot 7H_2O$, and 66 ml distilled water.

[7] Designated PCB-45 by Okamoto (13).

sample in suspension was mixed in Hanks' saline solution of this medium so as to contain serial concentrations.

After incubation at 37 C for 48 hours, samples of the incubation mixture were removed and the number of cells was determined by microscopy using crystal violet staining or a Coulter counter model B.

The test samples were prepared as follows: the bacterial cells of OK-431 or OK-432 suspension were collected by centrifugation, washed with physiologic saline, and finally resuspended in Hanks' saline solution.

In Vivo Antitumor Tests

The effect on life prolongation was examined using the following tumors: rat ascites hepatomas, such as AH-13, AH-44, AH-66, AH-66F, AH-130, AH-272, AH-414, AH-7974, AH-109A, AH-225A, and AH-41C; Yoshida sarcoma; nitrogen mustard-resistant[8] sublines of Yoshida sarcoma GY and GH, whose resistance indices[9] were 100; Ehrlich carcinoma; and mouse leukemia L1210. The tumor cells of rat ascites hepatoma or Yoshida sarcoma, or its nitrogen mustard-resistant sublines, were suspended in sterile physiologic saline to make about 10^6 cells/ml and were then inoculated ip in Donryu rats. Except when otherwise indicated, 30-150 KE/kg of OK-431 or OK-432 was injected ip daily for 10–15 days starting 24 or 72 hours after tumor cell inoculation. In the case of pretreatment, 30–50 KE/kg of OK-432 was given ip for 7 successive days to normal healthy rats and 10^6 cells of AH-13 were inoculated ip 8 days after the last injection. In most cases 60-day survival was observed. The following is the testing schedule for Ehrlich carcinoma. *Inoculum size:* ip, 1×10^6 cells per mouse; sc, 5×10^6 cells per mouse. *Administration:* dose—50–100 KE/kg/day (ip-ip)[10] or 200 KE/kg/day (sc-ip, sc-iv); treatment period—3–5 days, starting 24 hours (ip-ip) or 72 hours (sc-ip, sc-iv) after inoculation.

For sc inoculated Ehrlich carcinoma, the solid tumor mass at the inoculation site was removed and weighed 15 days after inoculation. For mouse leukemia L1210, 10^5 cells per mouse were injected ip and 250–1000 KE/kg of OK-432 was given daily ip from the day after inoculation until the death of the animal.

RESULTS

Toxicity of OK-432

The acute toxic effects of OK-432 in various animals are shown in table 1.

[8] Nitrogen mustard: NSC-762; diethylamine, 2,2'-dichloro-*N*-methyl-, hydrochloride; HN2.

[9] Resistance index = MEC of resistant cells/MEC of original Yoshida sarcoma cells. MEC = the minimum effective concentration of nitrogen mustard which causes abnormal mitosis in more than 50% of the mitotic cells.

[10] Ip-ip = ip inoculation and ip treatment; sc-ip = sc inoculation and ip treatment; sc-iv = sc inoculation and iv treatment.

TABLE 1.—Acute toxicity of OK-432 in various animals

Animals	Sex	LD50 (KE/kg)	
		Ip treatment	Iv treatment
Mice	F	1380 (1280–1470)*	271 (260–290)
	M	1290 (1210–1360)	230 (210–250)
Rats	F	1690 (1530–1850)	260 (210–320)
	M	1750 (1640–1850)	250 (190–325)
Rabbits	F	—	110–160
Guinea pigs	F	—	100
Cats	F	—	75–125
Dogs	F	—	20–100
Monkeys	F	<200	17.5

*95% confidence limits.

For subacute toxicity tests, rats were given 12.5-100 KE/day ip for 4 weeks. At a dose of 12.5 KE/day (76 KE/kg/day) there were no significant changes in erythrocyte counts, leukocyte counts, serum hemoglobin levels, hematocrit values, and serum transaminase activity. Slight anemia with an increase in the leukocyte count gradually developed in rats given 25–100 KE/day (149–575 KE/kg/day). On the other hand, daily iv administration of 6.25–12.5 KE/day (54–107 KE/kg/day) in rats, caused more pronounced anemic symptoms with a decrease in erythrocyte counts, serum hemoglobin levels, and hematocrit values, while serum transaminase activity and leukocyte counts were not altered significantly. Slight nephritis was noted histopathologically in approximately half the rats given 12.5 KE/day that were killed at the end of the treatment period. Blood urea nitrogen (BUN) values did not change significantly. Iv injection of 25 KE (222 KE/kg) to rats was fatal with most of the animals dying during the administration period.

In rabbits and dogs treated iv for 4 weeks, similar changes in the blood studies were observed. Moderate nephritis was noted in all rabbits given more than 10 KE/day iv (3.8 KE/kg/day) and in all dogs given more than 200 KE/day (11.1 KE/kg/day), with no change in the BUN values.

In all animals tested no injury to bone marrow was observed histopathologically.

In rabbits given 25 KE/day sc (10 KE/kg/day) for 2 months, serum protein levels, especially gamma globulin, increased considerably from 2 weeks after the start of treatment, and at the same time antibody formation (by agglutinin test) against *Streptococcus hemolyticus* (Su strain) increased, although the antibody titer was relatively low (not more than 1:64).

Pyrexia in rabbits given OK-432 iv in various doses was less severe than that caused by endotoxin of gram-negative bacteria. The minimum pyrogenic dose in rabbits was 0.32 KE given iv.

Antitumor Effect

1. In vitro effect.—As shown in figure 1, the 50% inhibition concentration (IC50)[11] of OK-431 against the cells of Yoshida sarcoma and AH-13 was 0.25–0.50 KE/ml, and 1 KE/ml produced almost complete inhibition of the growth of Yoshida sarcoma cells. There was no significant difference in the growth inhibitory effect between OK-432 and OK-431. When the growth inhibitory activity of OK-432 on Yoshida sarcoma and the nitrogen mustard-resistant sublines, GY and GH, was examined in vitro, OK-432 revealed almost similar IC50 values against these three tumor cells, although it proved to be most effective against one of the nitrogen mustard-resistant sublines, GH,

[11] IC50 is the concentration of a drug which reduces the tumor cell count to 50% of the control.

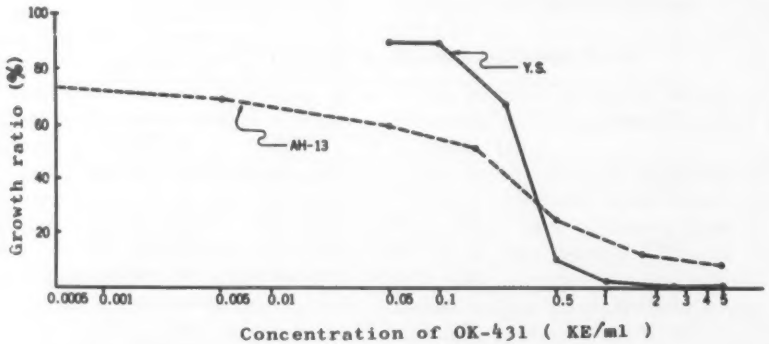

FIGURE 1.—Inhibitory effect of OK-431 on the growth of Yoshida sarcoma (Y.S.) or ascites hepatoma AH-13 in vitro. Number of Yoshida sarcoma and AH-13 cells in the control growth in vitro ($\times 10^4$): *Yoshida sarcoma*—initial, 4.4; after 48 hours, 27.0. *AH-13*—initial, 4.9; after 48 hours, 17.0.

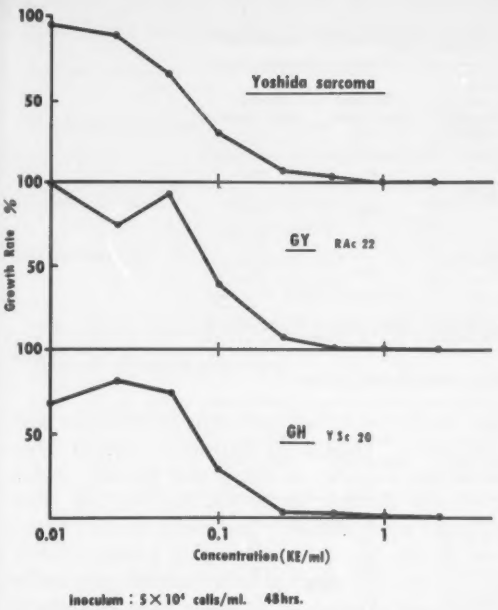

FIGURE 2.—Growth inhibition of Yoshida sarcoma and two nitrogen mustard-resistant sublines of Yoshida sarcoma by OK-432 in vitro.

in in vivo testing. These results, shown in figure 2, indicate that OK-432 has a direct growth-inhibitory effect against tumor cells.

2. In vivo effect of OK-431.—OK-431 had a significant antitumor effect on AH-13 and AH-66 as shown in table 2. From our experiences with alkylating agents in these tumor lines, it is clear that although AH-13 was sensitive to alkylating agents, AH-66 was rather an insensitive strain. Except for one animal in the group of AH-66-bearing rats given 100 KE/kg that died from pneumonia, all the animals given 20, 30, or 100 KE/kg survived during the 60-day observation period and no tumor cells were noted in the ascites. Sc injection of OK-431 was also somewhat effective in rats bearing AH-13. Examination of the antitumor effect of OK-432 on rats bearing AH-13 and Yoshida sarcoma showed that both OK-431 and OK-432 had almost the same effect. When OK-431 was given ip or iv at a dose of 200 KE/kg/day for 3 days, the inhibition rate against Ehrlich carcinoma was about 60%–70%, showing the same degree of inhibition as mitomycin C (NSC-26980) (2 mg/kg/day). The antitumor activity of OK-431 against Ehrlich ascites carcinoma was even better than that of mitomycin C (table 3).

3. In vivo effect of OK-432.—From our previous studies on the effect of various alkylating agents on rat tumors, Yoshida sarcoma, AH-13, AH-130, AH-272, and AH-66F were sensitive to the agents; AH-109A, AH-225A, and AH-414 were medially sensitive; and AH-41C, AH-66, AH-7974, and AH-44 were resistant. OK-432 was somewhat effective in AH-414 and AH-41C but little effect was noted against Yoshida sarcoma, one of the nitrogen mustard-resistant sublines, GY, and AH-109A and AH-44 by the ip-ip system. In rats bearing AH-109A or Yoshida sarcoma, the tumor cells grew again after the end of administration and the rats finally died from tumor invasion into various tissues. It should be noted that OK-432

TABLE 2.—Effect of OK–431 on rat ascites hepatoma AH–13 and AH–66*

Tumor	Lifespan of control animals (days)	Dose (KE/kg×30)	30-day survivors (%)		60-day survivors (%)	
			Ip treatment	Sc treatment	Ip treatment	Sc treatment
AH–13	6–10	100	100	40	100	40
		33	100	20	100	20
		20	100	40	100	30
AH–66	12–17	100	100	—	90	—
		33	100	—	100	—
		20	100	—	100	—

*OK–431 was given ip or sc from the day after ip inoculation of 10^6 cells per rat, daily for 30 days. 10 rats per group.

TABLE 3.—Effect of OK–431 and OK–432 on Ehrlich ascites carcinoma*

Experiment No.	Preparation	Lifespan of control animals (days)	30-day survivors (%)	60-day survivors (%)
1	OK–431 (100 KE/kg)	11–18	90	70
	Mitomycin C (2 mg/kg)		100	20
2	OK–431 (50 KE/kg)	8–17	90	30
	OK–432 (50 KE/kg)		70	50

*Each preparation was given ip daily for 3 days (expt 1) or 4 days (expt 2) starting 24 hours after ip inoculation of 10^6 cells per mouse. 10 mice per group.

TABLE 4.—Effect of OK–432 on various rat tumors*

Tumor	Lifespan of control animals (days)	Dose (KE/kg× No. of doses)	30-day survivors (%)	60-day survivors (%)
Yoshida sarcoma	6–12	150×15	10	10
		100×10†	0	0
		50×15	20	10
		30×15	30	20
GH sarcoma‡	8–12	150×15	60	60
		100×10†	80	80
		50×15	40	40
		30×15	0	0
GY sarcoma‡	7–11	150×15	10	10
		50×15	10	10
		30×15	20	20
AH–13	6–12	150×15	100	100
		100×10†	100	100
		50×15	100	100
		30×15	100	100
		10×15	70	70
		5×15	70	70
		1×15	0	0
AH–414	18–27	100×10†	80	80
AH–109A	9–12	100×10†	20	20
AH–41C	12–20	100×10†	70	50
AH–44	6–9	100×10†	30	30
AH–7974	10–13	100×10†	0	0
AH–66	6–9	100×10†	20	20
AH–66F	6–9	100×10†	0	0
AH–272	6–12	100×10†	20	20
AH–225A	12–13	100×10†	0	0
AH–130	10–13	100×10†	0	0

*OK–432 was given ip daily for 10 or 15 days from the day after ip inoculation of 10^6 tumor cells per rat. 10 rats per group.
†Treatment was started 3 days after inoculation. 6 rats per group.
‡One of the nitrogen mustard-resistant sublines of Yoshida sarcoma.

had an antitumor effect on rats bearing GH, the nitrogen mustard-resistant subline of Yoshida sarcoma, although it was less effective in the other nitrogen mustard-resistant subline, GY. These results are summarized in table 4.

The foregoing data were obtained by the ip-ip system. In a similar experiment in which only the route of administration was changed, both iv and sc injections of OK-432 were effective in AH-13, while no life-prolonging effect

TABLE 5.—Comparison of the effect of OK–432 on AH–13 and AH–44 with various combinations of route of administration and inoculation*

Route of inoculation	Tumor	Dose (KE/kg×10)	30-day survivors (%)			60-day survivors (%)		
			Ip treatment	Iv treatment	Sc treatment	Ip treatment	Iv treatment	Sc treatment
Ip (10^6 cells/rat)	AH–13 (6–12)†	100	—	70	30	—	50	30
	AH–44 (6–9)†	100	—	0	20	—	0	0
Iv (10^7 cells/rat)	AH–13 (8–12)†	100	40	20	0	40	20	0
	AH–44 (6–18)†	100	80	100	100	80	100	100

*OK–432 was given ip, iv, or sc daily for 10 days starting 3 days after ip or iv inoculation of 10^6 or 10^7 cells per rat. 6 rats per group.
†Lifespan of control rats (days).

was noted by iv injection and sc injection was only slightly effective in AH-44 (table 5). When OK-432 was given to rats inoculated iv with 10^7 cells of AH-13 or AH-44, however, no antitumor effect was noted in those bearing AH-13 treated ip, iv, or sc, although significant effect was demonstrated in those bearing AH-44 treated by each route of administration (table 5).

A pronounced prolongation of lifespan was observed in rats inoculated with AH-13 cells on the eighth day after seven successive daily pretreatments with OK-432. Therefore, it can be said that OK-432 has host-mediated antitumor activity as well as a direct cytocidal effect estimated by in vitro testing. This result is shown in figure 3. As can be seen in table 3, OK-432 was as effective as OK-431 in Ehrlich ascites carcinoma.

OK-432 was ineffective in mouse leukemia L1210 even after administration of a large dose (250–1000 KE/kg/day) for 7 days.

DISCUSSION

In these studies we have confirmed the direct effect of OK-432 on tumor cells in vitro. To examine the cell-damaging activity of *Streptococcus hemolyticus*, Shimizu et al (17) and Hatano (18) tried contacting living streptococcal cells (Su strain) with the cultured tumor cells. It was found that a large amount of RNA was released from the tumor cells and their cyto-

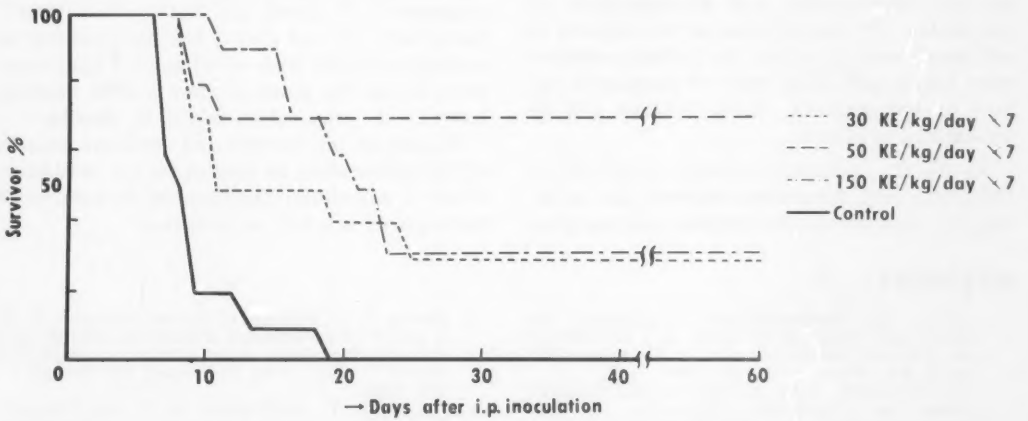

FIGURE 3.—Effect of OK-432 pretreatment on the growth of ascites hepatoma AH-13. (OK-432 was given ip for 7 successive days to normal rats and 10^6 AH-13 cells were inoculated ip.)

plasms were stained less intensely by toluidine blue than those of the controls.

Kurata et al (19) observed that RNA synthesis of ascites hepatoma AH-130 cells, examined by the incorporation of ^{3}H-uridine into the RNA fraction of the cells, was suppressed by the addition of OK-432 in the incubation medium, though the incorporation of ^{3}H-phenylalanine into protein or the incorporation of ^{3}H-thymidine into the DNA fraction was not affected significantly by OK-432. It was observed that the incorporation of ^{3}H-uridine or ^{3}H-thymidine into Yoshida sarcoma or Ehrlich carcinoma cells was suppressed by the addition of OK-432 in vitro (unpublished data).

The morphologic study was then carried out in the process of degradation of the tumor cells by OK-432 through phase microscopic or electron microscopic observation. Within 1 hour after contact of the AH-13 cells with OK-431 in the rat peritoneal cavity, vacuolization and deformation of the cells were observed, and degradation of the cells proceeded further, resulting in appearance of naked nuclei (20). Cells of Yoshida sarcoma, incubated for 1–3 hours with OK–432 at 37 C, showed the following features: nuclei were condensed or swollen and cytoplasms adjacent to cell membranes became rough with swelling; free ribosomes were degenerative, disappearing partially; mitochondria were swollen considerably, showing degeneration and disappearance of the cristae. No degeneration or lytic figures of cell membranes to which the bacteria adhered were found, and there were no phagocytic figures of the bacteria. Such findings will be published elsewhere.

As for the antitumor activity of OK-431 or OK-432 in vivo, the authors reported that intratumoral injection caused necrosis and complete cure of VX2 carcinoma transplanted in rabbits, and that iv administration to C3H/He mice bearing spontaneous mammary tumors inhibited tumor growth and produced necrosis of the tumors (21). A considerable prolongation of lifespan was observed in mice bearing transplantable ascites mammary tumor (MM2) after the administration of OK-432 (unpublished data). These results indicated that OK-431 and OK-432 were effective not only in isologous experimental tumors but also in spontaneous tumors.

One of the interesting points in this study is that OK-432 was effective in a nitrogen mustard-resistant subline of Yoshida sarcoma. Also, OK-432 has a life-prolongation effect on rats pretreated with this preparation before ip inoculation of rat ascites hepatoma AH-13. Because this preparation has direct antitumor activity, which was proved by in vitro contact of the tumor cells with OK-432, it is important to note that both pretreatment and posttreatment of rats bearing ascites hepatoma with this preparation were effective, resulting in the prolongation of lifespan.

Rapaport et al (22–25) reported that heat-killed Group A hemolytic streptococci can induce a state of altered reactivity to skin homografts in guinea pigs. However, the mechanism of the host-mediated activity of OK-432 is still uncertain. Therefore, it is concluded that OK-432 prepared from *Streptococcus hemolyticus* possesses both direct and host-mediated antitumor activity, and that it has little toxicity in animals and very little effect against bone marrow. From this point of view clinical application of this preparation might be possible.

Studies on the biologic and serologic aspects of the preparation as well as on the antitumor effect of subcellular fractions of *Streptococcus hemolyticus* are now in progress.

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Increased Lifespan Using the Priming Dose Schedule Design in Carbazilquinone (NSC-134679) Treatment of Mouse Leukemia L1210[1, 2]

Marc J. Straus [3] and Abraham Goldin [4,5]

SUMMARY

This study was conducted to determine whether priming dose schedules of the new drug carbazilquinone could result in increased antileukemic effectiveness. CDF_1 mice were inoculated intraperitoneally with 10^5 L1210 ascites tumor cells. In one experiment groups of mice were treated with carbazilquinone on a primary screening schedule consisting of single-dose, weekly, and daily therapy starting 1 day after tumor inoculation. The longest surviving group treated with carbazilquinone received 2.0 mg/kg/week which increased the median survival time over control mice by 175%. On the dose schedules used in this experiment, carbazilquinone was as effective as methotrexate and somewhat less effective than cyclophosphamide. In a second experiment a number of geometrically spaced doses of carbazilquinone were used during the first treatment and each was followed by various geometrically spaced levels of regularly scheduled carbazilquinone at 4-day intervals. In a number of groups of mice which had received a high loading dose followed by lower doses there were cured animals. No mice treated on a constant dose schedule were cured. The results of this study suggest that the loading dose schedule can be fully incorporated early in the chemotherapeutic evaluation of new agents. The results also emphasize the importance of further investigation of new scheduling procedures.

[Cancer Chemother Rep 56:19–23, 1972]

A number of derivatives of aziridinyl benzoquinone have been synthesized and tested for antileukemic L1210 activity (1). Of these, carbazilquinone (fig 1) appeared to be the most effective in increasing the survival time of leukemic mice.[6] It was reported to be effective on continued, intermittent, or single intraperitoneal (ip) administration against L1210 leukemia (1). Carbazilquinone has also been reported to exert a therapeutic effect against four other transplantable tumors including a myeloid leukemia. In addition, it inhibited the growth of a methylcholanthrene-induced tumor and a spontaneous tumor in C_3H mice (2).

In view of previous studies in which the use of loading schedules of the antimetabolite methotrexate (3) and the alkylating agent

[1] Received May 27, 1971; revised Sept 2, 1971.

[2] Carbazilquinone: *p*-benzoquinone, 2,5-bis(1-aziridinyl)-3-(2-hydroxy-1-methoxyethyl)-6-methyl-, carbamate (ester). Supplied by Sankyo Co. Ltd., Tokyo, Japan.

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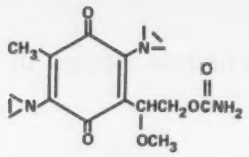
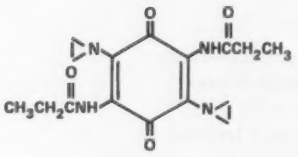
[5] The authors wish to acknowledge the technical assistance of Mrs. Icilda Riley, Mrs. Ernestine Gregory, and Miss Elizabeth Winans, and the secretarial assistance of Mrs. Sally Bernstein and Mrs. Linda Henry.

[6] Aziridinyl benzoquinones: NSC-18271 (propionamide, *N,N'*-[3,6-bis(1-aziridinyl)-*p*-benzoquinon-2,5-ylene]bis-) and NSC-17261 (*p*-benzoquinone, 2,5-bis-(1-aziridinyl)-3,6-dipropoxy-).

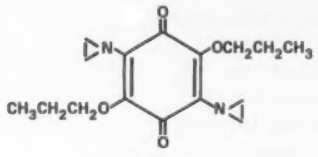


NSC–134679

CARBAZILQUINONE



NSC–18271



NSC–17261

FIGURE 1.—Carbazilquinone (NSC-134679) and two structurally related aziridinyl benzoquinones (NSC-18271 and NSC-17261).

cyclophosphamide (unpublished data) resulted in increased lifespan of L1210 mice, it seemed worthwhile to extend such studies to a new agent with demonstrated antileukemic activity.[7]

MATERIALS AND METHODS

Stock tumor of lymphoid leukemia L1210 was carried ip in DBA/2 male mice. Ascites tumor cells were counted in a hemocytometer, diluted in Hanks' Balanced Salt Solution to 1×10^6 cells/ml, and the suspension kept in an ice bath. One $\times 10^5$ cells (0.1 ml) of tumor cell suspension were injected ip into CDF_1 (BALB/c AnCr $\times$ DBA/2Cr)F_1 male mice weighing 19–25 g. Mice were randomized and housed in plastic cages in a constant temperature facility and provided with water and laboratory chow ad libitum.

Carbazilquinone was freshly prepared at each injection period and refrigerated in an opaque jar. It was suspended in a steroid suspending vehicle (containing sodium chloride, sodium carboxymethylcellulose, polysorbate, and benzyl alcohol) with the aid of a magnetic stirring bar. Serial dilutions of the drug were prepared at 0.5 intervals and injected over a range of 32.0 to 0.03 mg/kg. After preparation, the drug was immediately given ip since it tended to settle rapidly.

Cyclophosphamide was dissolved in 0.85% saline and injected ip. It was freshly prepared at each injection period. Methotrexate was dissolved in 2% sodium bicarbonate and injected ip. It was prepared every 4 days and refrigerated. All drugs were administered at a volume of 0.01 ml/g body weight.

RESULTS

The experiments are summarized in tables 1 and 2. In both experiments the median survival time (MST) of the untreated control mice was 8.0 days. On a single-treatment schedule with carbazilquinone (Day 1 only) the longest MST (13.0 days) was observed at a dose of 2.0 mg/kg (table 1). However, one of eight mice which had received a higher dose (4.0 mg/kg) on Day 1 was cured. A dose of 8.0 mg/kg was toxic and the MST was reduced to 6.0 days. Of the groups of mice treated with carbazilquinone weekly from Day 1 to death, the longest MST (22.0 days) was at 2.0 mg/kg weekly. On a daily schedule the group which survived longest (MST, 16.0 days) had received 0.5 mg/kg/day. Thus, the longest surviving group had received carbazilquinone weekly.

On the dose schedules used, carbazilquinone was as effective as methotrexate and less effective than cyclophosphamide. When methotrexate was administered as a single dose or on a weekly or daily schedule the longest MST (21.0 days) was observed on a daily schedule (1.01 mg/kg/day). Cyclophosphamide was similarly tested and four of eight mice which had received a single dose of 200 mg/kg on Day 1 were cured. The MST of mice that received

[7] Methotrexate: NSC-740; glutamic acid, *N*-[*p*-[[(2,4 - diamino - 6 - pteridinyl)methyl]methylamino]-benzoyl]-.

Cyclophosphamide: NSC-26271: 2*H*-1,3,2-ozazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate; Cytoxan.

TABLE 1.—Comparison of carbazilquinone, methotrexate, and cyclophosphamide in the treatment of mouse leukemia L1210*

Agent dose (mg/kg)	Ip treatment schedule	Median survival time (days)	Percent increase over controls
Carbazilquinone			
32.0	Day 1 only	4.5	0
16.0		5.0	0
8.0		6.0	0
4.0		11.0†	37.5
2.0		13.0	62.5
1.0		11.0	37.5
0.5		9.5	18.8
0.25		9.0	12.5
0.125		8.5	6.3
32.0	Weekly, Day 1 to death	4.0	0
16.0		5.0	0
8.0		9.5	18.8
4.0		12.0	50.0
2.0		22.0	175.0
1.0		12.0	50.0
0.5		10.0	25.0
0.25		9.0	12.5
0.125		9.0	12.5
0.06		8.0	0
32.0	Daily, Day 1 to death	3.0	0
16.0		4.0	0
8.0		5.0	0
4.0		6.5	0
2.0		8.0	0
1.0		11.5	43.8
0.5		16.0	100
0.25		11.0	37.5
0.125		10.0	25.0
0.06		9.5	18.8
0.03		8.5	6.3
Methotrexate			
100	Day 1 only	7.0	0
60		11.0	37.5
36		10.0	25.0
21.6		11.0	37.5
100	Weekly, Day 1 to death	5.0	0
60		12.0	50
36		12.0	50
21.6		12.0	50
1.68	Daily, Day 1 to death	15.0	87.5
1.01		21.0	162.5
0.60		17.5	118.7
Cyclophosphamide			
400.0	Day 1 only	6.0	0
200.0		35.0‡	337.5
100.0		13.0	62.5
50.0		10.0	25.0
400.0	Weekly, Day 1 to death	11.0	37.5
200.0		23.5	193.7
100.0		16.5	106.3
50.0		12.5	56.2
200.0	Daily, Day 1 to death	7.0	0
100.0		17.0	112.5
50.0		18.5	131.2
25.0		12.0	50
Untreated controls		8.0	

*CDF_1 mice (19–25 g) were inoculated with 10^5 L1210 ascites tumor cells ip, Day 0. There were 8 mice per group. Median survival time of untreated control mice was 8.0 days. Cured mice survived longer than 90 days but were recorded as having lived 60 days.
†1 cure (survived >90 days). ‡4 cures.

200 mg/kg weekly from Day 1 to death was 23.5 days.

In a second experiment a comparison was made between the therapeutic effectiveness of a constant dose schedule and a loading dose schedule. In this experiment the interval of administration of carbazilquinone was 4 days (q4d) (table 2). For a constant dose schedule, the group that received 1.40 mg/kg q4d survived the longest (MST, 25.5 days). The higher dose level of 2.0 mg/kg q4d was apparently toxic since the MST was reduced to 17.5 days. The maximum survival times observed with the loading dose schedule were considerably greater than those observed with a constant dose schedule. The two most effective schedules included a priming dose of 2.86 mg/kg on Day 1 and 0.69 mg/kg q4d from Day 5, or 2.0 mg/kg initially and 1.40 mg/kg q4d from Day 5. In both groups the MST was 60.0 days and five of eight mice were cured.[8] There were also two of eight animals cured in each of three groups which had received a loading dose of 2.86 mg/kg followed by either 0.98 mg/kg, 0.48 mg/kg, or 0.33 mg/kg q4d.

There were six additional mice cured in groups which had received 2.00 mg/kg initially, and three cured mice in groups which had been treated with 1.40 mg/kg on Day 1 followed by lower doses. Of all the mice that had received constant dose schedules, or an initial dose lower than subsequent doses (groups above the diagonal line in table 2), there was only one mouse which was cured.

DISCUSSION

The current studies corroborate the findings of Arakawa et al (1) that treatment with carbazilquinone increases the lifespan of L1210 leukemic mice on a number of schedules of administration. Evidence that the effectiveness of the drug is schedule dependent is provided by the current observation that a weekly schedule was more effective than single or daily treatment. On these standard constant dose schedules carbazilquinone was as effective as methotrexate but somewhat less effective than cyclophosphamide.

[8] These mice survived longer than 90 days, but were recorded as having lived 60 days.

TABLE 2.—Effect of loading dose schedules of carbazilquinone on survival time in mouse leukemia L1210*

Carbazilquinone (mg/kg, q4d, Day 5 to death)	Median survival time†						
	Initial dose of carbazilquinone on Day 1 (mg/kg)						
	2.86	2.00	1.40	0.98	0.69	0.48	0.33
2.86	8.5				10	10.5	12
2.00	11.5	17.5	18.5	17	13.5	17	19.5
1.40	9.5	60 (5)	25.5	26 (1)	18	20	20
0.98	24.5 (2)	23.5	16.5 (1)	18	18	16	14.5
0.69	60 (5)	21 (3)	18.5 (2)	15.5	14	13	13
0.48	21 (2)	22	17	13.5	13	13	12
0.33	14 (2)	23 (3)	16	13.5	13		10.5

*CDF_1 mice (19–25 g) were inoculated with 10^5 L1210 ascites cells ip, Day 0. There were 8 mice per group. Median survival time (MST) of untreated control mice was 8.0 days. Cured mice survived longer than 90 days but were recorded as having lived 60 days. Treatment was ended on Day 40. The MST of groups of mice which received the constant dose schedules are joined by a diagonal line.

†Numbers in parentheses indicate the number of mice cured.

In addition, these studies demonstrate that the chemotherapeutic effectiveness of carbazilquinone can be increased significantly by the use of a loading dose schedule design. With a loading dose schedule involving a single high dose followed by multiple treatments with low doses q4d, a high percentage of the animals survived, apparently without evidence of leukemia.

It would appear that the loading dose schedule optimizes tumor cell kill. In earlier studies it had been reported that the loading doses of methotrexate increased the survival time of L1210 mice (3). It was suggested that the percentage of tumor cells killed by methotrexate, a cell-cycle stage-specific agent, increases as the tumor cell population decreases. By lowering the tumor cell population the initial loading doses enable subsequent lower doses to kill more effectively. Other studies with cyclophosphamide, a cell-cycle stage-nonspecific agent, have suggested that when the tumor is very large the percentage of tumor cells killed by the drug is diminished. In certain schedules, loading doses increased the effectiveness of cyclophosphamide (unpublished data).

In the studies using methotrexate and cyclophosphamide the initial loading dose of the optimal schedules approximated the optimal single dose. This is also apparent in the current study.

This study along with the previous studies involving methotrexate (3) and cyclophosphamide (unpublished data) indicate a possibility that the observation of improved therapy with the loading dose procedure may be a generalized phenomenon.

With new drugs such as carbazilquinone the loading dose design may be used effectively based on information obtained in the standard schedule-dependency dose-response experiment of a primary screen. These ordinarily include single, daily, weekly, and other intermittent schedules (4). In designing an effective loading dose schedule, it might be anticipated that the optimal initial dose will approximate the optimal single dose. For maximum effectiveness it would appear to be desirable to select a treatment interval which does not exceed the increase in lifespan obtained with the optimal dose on single treatment.

Aside from the consideration of including loading dose schedule designs early in the chemotherapy screening process of new agents, it may be worthwhile to utilize the loading dose design to re-evaluate drugs which, though active, had not been considered sufficiently active based on standard tests to warrant clinical interest. Other active benzoquinones (5) (fig 1) are currently being re-evaluated.

Other studies could be conducted to determine in what manner alteration of dose levels of drug other than the initial dose may influence chemotherapeutic effectiveness. A more diffi-

cult but useful study might include a schedule with varying times between administration of individual doses of drug. The improved therapy obtained to date with the loading dose procedure should encourage further investigation of new scheduling designs.

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Effect of Day of Dosage Omission on Survival Time in the Regularly Scheduled Methotrexate (NSC-740) Treatment of Leukemia L1210[1,2]

Marc J. Straus[3] and Abraham Goldin[4,5]

SUMMARY

This study was conducted to determine the effect on survival time of altering a regular schedule of methotrexate therapy for L1210 mouse leukemia by variously omitting one of the scheduled doses. From the two schedules of methotrexate studied it appears that survival time is a monotone increasing function of the omission time. These results support prior studies which suggested that the percent of tumor cell kill of methotrexate may decrease as the population of tumor cells increases. The earlier a dose of scheduled methotrexate is omitted, the greater are the number of subsequent doses which must act against an increased population, thereby decreasing survival time. This study emphasizes the importance of early administration of an effective priming dose.

[Cancer Chemother Rep 56:25–30, 1972]

In previous studies involving methotrexate treatment of mouse leukemia L1210, it was demonstrated that longer survival occurred when treatment was started with a high priming dose of methotrexate followed by lower doses (1). Other data also suggested that methotrexate, as a cell-cycle stage-specific agent, kills a smaller percent of tumor cells as the population of tumor cells increases (1,2). Therefore, the priming dose of methotrexate lowers the population of tumor cells sufficiently for subsequent doses of methotrexate to kill more effectively.

In other experiments using leukemia L1210, it has been shown that when a high dose of cyclophosphamide was substituted for a regularly scheduled dose of methotrexate, the earlier the substitution the longer the survival (2). This effect was noted when the scheduled interval between doses of methotrexate was 2 or 4 days. The high priming dose[6] of cyclophosphamide,[7] when given early, also allowed a greater number of subsequent doses of methotrexate to exert increased effect by acting against a reduced population. When the priming dose of cyclophosphamide was as effective as the dose of methotrexate being replaced, the advantage in early priming dose therapy disappeared. In fact, in one experiment (2) where the priming dose was slightly less effective than the regu-

[1] Received May 28, 1971; revised Sept 8, 1971.

[2] Methotrexate: glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-. Supplied by Lederle Laboratories, Pearl River, NY.

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[5] Statistical analysis was given by the Division of Biostatistics, Washington University School of Medicine, St. Louis, Mo. Technical assistance was given by Mrs. Ernestine Gregory.

[6] "Priming dose" is not used here in the pharmacologic sense, but means a single dose of any drug administered sequentially with a series of doses of the test drug (methotrexate in this experiment). The single priming dose may occur anywhere in the sequence. It may be high (loading dose) or it may be zero dose (omitted) as in this experiment. It may be the same drug as the test drug.

[7] Cyclophosphamide: NSC-26271; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate.

larly scheduled dose of methotrexate, it appeared to be disadvantageous to administer the priming dose early in the course of therapy.

To examine the latter phenomenon more critically, the current study was conducted in which the priming dose was reduced to zero (drug omission). In the study, one of the regularly scheduled doses of methotrexate was omitted at various times during the course of therapy.

MATERIALS AND METHODS

Stock tumor of lymphoid leukemia L1210 was carried intraperitoneally (ip) in DBA/2 mice. Ascites tumor cells were counted in a hemocytometer, diluted in Hanks' Balanced Salt Solution to 1×10^6 cells/ml, and the suspension kept in an ice bath. A total of 1×10^5 cells (0.1 ml) of tumor cell suspension was injected ip into CDF_1 (BALB/c AnCr $\times$ DBA/2Cr)F_1 male mice, weighing 19–25 g. Mice were randomized and housed in plastic cages in a constant temperature facility and provided with water and laboratory chow ad libitum.

Methotrexate (Lederle lot No. 1260 $\times$ 8105) was prepared every 4 days and refrigerated in an opaque jar. It was dissolved in 2% sodium bicarbonate and injected ip at a volume of 0.01 mg/g body weight.

RESULTS

In the first part of the experiment (fig 1) the mean survival time (MST) of untreated control mice was 10 days. A single dose of methotrexate of 4 mg/kg administered on Day 2 increased the MST to 11.7 days. The basic

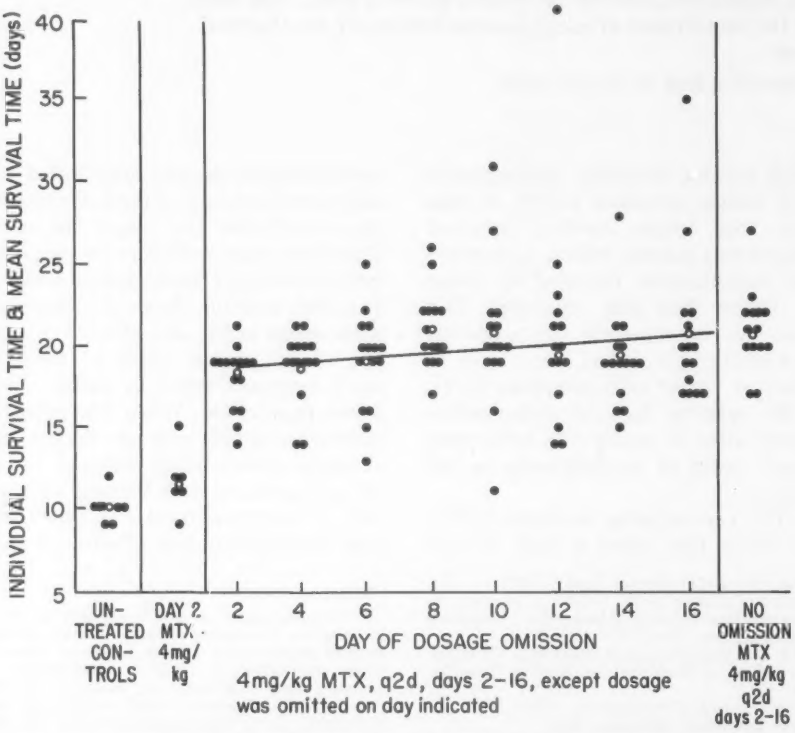

FIGURE 1.—CDF_1 mice received inoculations of 10^5 L1210 ascites cells on Day 0. Closed circles represent individual survival times of mice. Open circles represent mean survival times of the respective groups.

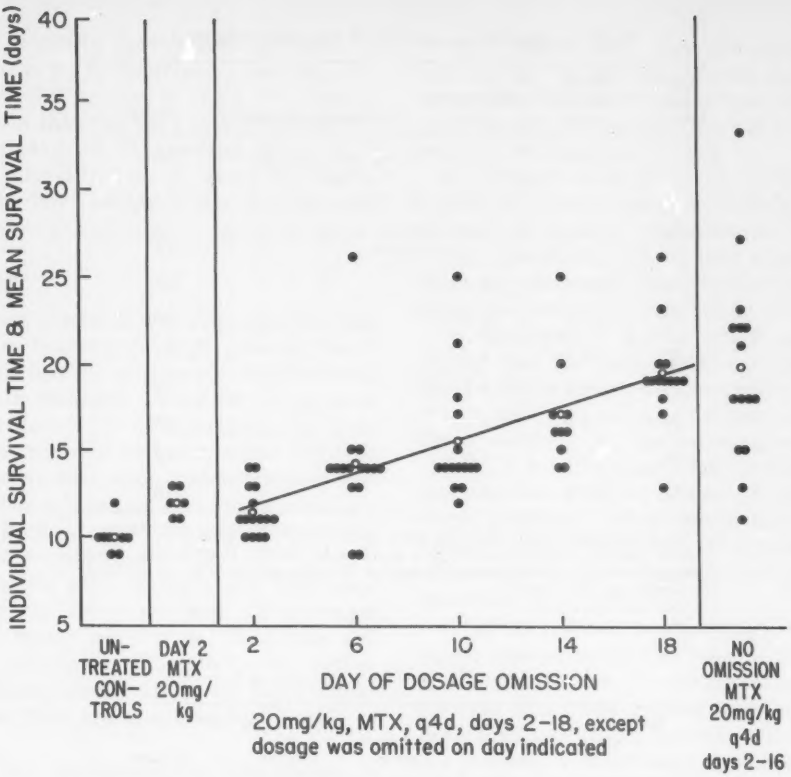

FIGURE 2.—CDF_1 mice received inoculations of 10^5 L1210 ascites cells on Day 0. Closed circles represent individual survival times of mice. Open circles represent mean survival times of the respective groups.

group of mice received methotrexate, 4 mg/kg, every other day on Days 2–16 after leukemic inoculation. The MST in this group was 21 days. In the other groups of mice a dose of methotrexate was omitted on the day indicated.

Of the groups of mice scheduled to receive methotrexate at a dose of 4 mg/kg every other day on Days 2–16, the group in which methotrexate was omitted earliest (Day 2) had the lowest MST (18.1 days). The MST of the group with the next earliest dosage omission (Day 4) was the next lowest (18.7 days). The group with the dose omitted the latest (Day 16) was among those showing the longest survival time (MST, 20.7 days). A plot of the MSTs suggests that in this experiment there was some advantage in delaying the day of dosage omission.

In the second part of the experiment (fig 2) the basic group of mice received a considerably higher dose of methotrexate, 20 mg/kg, every 4 days on Days 2–18. Its MST was 19.7 days. A single dose of methotrexate, 20 mg/kg, administered on Day 2, increased the MST to 12 days.

Of the groups with a dose of methotrexate omitted, again the group with the earliest omission (Day 2) had the lowest MST (11.5 days). The MST of the group with the next earliest dose of methotrexate omitted (Day 6) was the second lowest (14.1 days). With each delay in dosage omission there was an increase in MST. When the Day 18 dose was omitted, the MST was 19.3 days. A plot of MSTs indicates a

TABLE 1.—Statistical analysis of the data in figure 1

Day of omission (S)	Mean survival time	Standard error	Comparison of means*	P value
2	18.1	0.5	S_2, S_4, S_6, S_8, S_{10}, S_{12}, S_{14}, S_{16}	NS†
			$S_4 \rightarrow S_{16}$	$P<0.04$
4	18.7	0.5	S_4, S_6, S_8, S_{10}, S_{12}, S_{14}, S_{16}	NS
			$S_8 \rightarrow S_{16}$	NS
6	19.1	0.8		NS
8	20.9	0.6		NS
10	20.7	1.2		NS
12	20.7	1.7		NS
14	19.2	0.8		NS
16	20.7	1.2		NS
N‡	21.0	0.7		NS

*S_2 is not significantly different from each of the other groups in which a dose of methotrexate is omitted. S_2 is significantly smaller than the combined groups $S_4 \rightarrow S_{16}$. Similarly, testing S_4 against the other groups or $S_6 \rightarrow_{16}$ revealed no significant differences.
†NS = not significant.
‡S_N = group with no omission.

TABLE 2.—Statistical analysis of the data in figure 2

Day of omission (S)	Mean survival time	Standard error	Comparison of means*	P value
2	11.5	0.4	S_6–S_N	$P<0.01$
6	14.1	1.0	S_{10}	NS†
			S_{14}–S_N	$P<0.01$
10	15.5	0.9	S_{14}	NS
			S_{18}–S_N	$P<0.01$
14	17.0	1.0	S_{18}–S_N	$P<0.05$
18	19.3	0.8	S_N	NS
N‡	19.7	1.4		

*S_6–S_N denotes that S_2 is significantly less than each of S_6, S_{10}, S_{14}, S_{18}, and S_N with $P<0.01$. S_{18} and S_N are not significantly different. The results are obtained by the least significant difference test based on log survival time.
†NS = not significant.
‡S_N = group with no omission.

significant advantage in delaying dosage omission.

A statistical analysis of the data in figures 1 and 2 is presented in tables 1 and 2 respectively. To facilitate the necessary statistical assumptions, the survival data were transformed logarithmically. The analysis on variance was performed on both experimental results. It is indicated that for figure 1 there exists no statistically significant difference between eight means (table 1). However, for figure 2, six MSTs were significantly different at $P < 0.01$ (table 2).

In table 1, a linear comparison based on the

orthogonal contrasts showed that the mean of the first group, S_2, is significantly less than the mean of the other groups, $S_4 \rightarrow S_{16}$ ($P < 0.04$). This suggests that the MST is a monotone increasing function of the omission time. It is clear from table 2 that on the every-fourth-day schedule, the MST increases significantly with a delay in dosage omission.

DISCUSSION

In previous studies it was demonstrated that loading dose therapy (a high priming dose) could lead to further increases in survival time of mice with leukemia L1210 for drugs such as methotrexate (1), cyclophosphamide (unpublished data), and carbazilquinone (3).[8] It was also shown that when a high priming dose of a drug was substituted early for a regularly scheduled dose of methotrexate, therapeutic effectiveness was increased (2).

These studies with methotrexate (1,2) suggested that the agent produces an increased percent of tumor cell kill as the tumor cell population decreases. Therefore, the earlier a high priming dose is given, the more subsequent doses there are to act against a reduced population.

The current studies support this conclusion in that early omission of a dose of methotrexate reduced therapeutic effectiveness. It is considered that the earlier a dose is omitted, the less effective are the subsequent doses because they must act against an increased population.

Although statistical analysis of the data from the every-other-day schedule (table 1) suggested the monotone relationship of survival time and day of omission, there were no significant differences among individual groups as there were among groups in table 2. The every-fourth-day schedule better demonstrates the need for maximal delay in dosage omission perhaps because fewer and higher doses were given on that schedule than on the every-other-day schedule. On the latter schedule no group of mice missed more than one of eight doses of 4 mg/kg. On the every-fourth-day schedule, one of five doses of 20 mg/kg was omitted. The first group, S_2, on an every-fourth-day schedule, received no drug until Day 6.

A corollary which is borne out by the data is that at nontoxic levels it is disadvantageous to omit any dose of methotrexate. Venditti et al have previously shown that maximum survival time occurred when methotrexate treatment was continued indefinitely (4).

In the previous studies (2) it was demonstrated that the more effective a dose of drug was in single treatment the greater the effect it also had in increasing lifespan when introduced early in the course of therapy. Conversely, it would appear from the present study that the less effective a dose of drug was in single treatment (zero dose in this case), the greater was the net result with respect to loss of therapeutic effectiveness, especially in early omission.

Since the trend of protocol designs in clinical cancer chemotherapy has been in the direction of using more complex schedules involving individual drugs and greater numbers of drugs in combination (5–9), laboratory studies which are designed to determine more effective schedules and sequences of administration of chemotherapeutic agents have increasing relevance. The current series of studies (1,2) emphasize the importance of the effective level of the priming dose. Where the priming dose was highly effective there was considerable advantage to therapy in its early administration. Where the priming dose was reduced or omitted early in the scheduling there was a pronounced disadvantage to therapy. In general then, the importance of starting maximally available effective regimens of drugs as early as possible in the course of therapy with a single drug or combinations of drugs is indicated.

Since methotrexate may differ kinetically from other cycle-specific agents in its mode of action (10), similar studies with other cycle-specific agents as well as cycle-nonspecific agents are warranted in order to determine whether these findings are generally applicable.

[8] Carbazilquinone: NSC-134679; *p*-benzoquinone, 2,5-bis(1-aziridinyl)-3-(2-hydroxy-1-methoxyethyl)-6-methyl-, carbamate (ester).

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Effect of Autoclaving on Molecular Weight Distribution and Chemotherapeutic Activity of Clinical Samples of Poly IC (NSC-120949) [1,2,3]

John J. Coffey, Stephanie A. Kelley, and Isidore Wodinsky [4]

SUMMARY

Autoclaving of clinical samples of poly IC for 15 minutes at 20-pound pressure led to a fall in the apparent molecular weight of the polymer and a broadening of the molecular weight distribution. Unautoclaved poly IC was largely excluded from a 1% agarose gel, while an autoclaved sample was measurably retained. The autoclaved and unautoclaved samples of poly IC were indistinguishable in their chemotherapeutic effects in BDF_1 mice bearing B_{16} melanoma.

[Cancer Chemother Rep 56:31–33, 1972]

The synthetic polyribonucleotide, poly IC, has shown biologic activity, both as an inducer of interferon production (1) and as a potential antitumor agent in a variety of systems (2–4). Although clinical trials of poly IC are in progress (5,6), it is apparent that toxicity is a serious problem with this drug (7–10).

Evaluation of the efficacy and toxicity of poly IC at both preclinical and clinical levels is considerably complicated by the heterogeneity of the polymer. The single strands of polyinosinic and polycytidylic acid used in preparation of poly IC for the clinical dose formulation employed by the National Cancer Institute, vary in molecular weight between approximately 1 and 2×10^5 (*see* Materials and Methods). When these single chains are annealed into a double-helical polymer, annealing of overlapping ends produces chains of many times the single-chain molecular weight, with the final product ranging in the tens of millions. Since polymerization and annealing proceed in random fashion, a polydisperse polymer mixture is formed, the composition of which may depend on the exact reaction conditions. Subsequent handling may also affect molecular weight: in particular, the autoclaving to which clinical preparations have sometimes been subjected can cause degradation or denaturation of the helical structure (11). Within certain limits, the molecular weight distribution of poly IC can affect its biologic properties. If the polymer is degraded substantially below a molecular weight of 10^6, interferon-inducing properties are diminished (12). Carleton et al (11) appear to have measured interferon induction by their poly IC preparations, but do not present data on the autoclaved samples. In any case, the relationship between interferon induction and antitumor activity is not clear. We have, therefore, attempted to determine whether autoclaving decreases the molecular weight to a significant extent, and whether any molecular weight changes observed can be correlated with changes in antitumor effect.

MATERIALS AND METHODS

The poly I and poly C used in the preparation

[1] Received June 28, 1971; revised Sept 15, 1971.
[2] Supported by contract PH43-65-61 from Chemotherapy, National Cancer Institute (NCI).
[3] Poly IC: 5′-inosinic acid, polymers, complex with 5′-cytidylic acid polymers (1:1); polyinosinic:polycytidylic acid. Supplied by the NCI in two lots, Nos. 12-01-004A (autoclaved) and 12-99-003, and prepared by Chas. Pfizer and Co., Inc., Maywood, NJ.
[4] Life Sciences Division, Arthur D. Little, Inc., Acorn Park, Cambridge, Mass.

of poly IC were from P-L Biochemicals, Inc., whose specifications indicate sedimentation coefficients of 10-15S for poly I and 8-13S for poly C. These values indicate molecular weights in the range of 1–2 $\times$ 10^5 for the single chains. The autoclaving step was carried out at 20-pound pressure (121 C) for 15 minutes.

The molecular weight distributions of the samples were examined by chromatography on agarose gel (13–15). These gels were the Bio-Gel A series obtained from Calbiochem. Although sold as suspensions presumably ready for use, the gels yielded reasonable flow rates only after extensive removal of fine particles. During the washing process, the gels were equilibrated with 0.01 M tris-hydrochloride, pH 7.0, 0.01 M $MgCl_2$, containing 0.2% sodium azide as a preservative. All further operations were carried out in this buffer.

Columns of 1 $\times$ 20 cm were prepared, and chromatography of 0.5-ml aliquots of the clinical dosage form was performed at flow rates of 0.2–0.3 ml/minute controlled by a Buchler Polystaltic pump. The column eluate was passed through a flow microcuvette, and the transmittance at 260 mμ was determined in a Zeiss PMQ II spectrophotometer and graphically recorded. Transmittance readings have been converted to absorbance for presentation in figure 1.

The chemotherapeutic activity of the autoclaved and unautoclaved lots of drug was assayed in female BDF_1 mice bearing B_{16} melanoma, by daily intraperitoneal injection of the drug on Days 1–9 after tumor implantation. Median survival times were calculated and the chemotherapeutic effect measured as the ratio of treated to control survival times (T/C).

RESULTS

Both preparations of poly IC, when chromatographed on Bio-Gel A 1.5 m (8% agarose; exclusion limit 1.5 $\times$ 10^6), were eluted as single peaks, the void volume of the column. With gels through A15 m (4% agarose; exclusion limit 1.5 $\times$ 10^7), both preparations were eluted as single peaks in a small volume, presumably the void volume. However, standards to determine the void volume of these columns are not available to us.

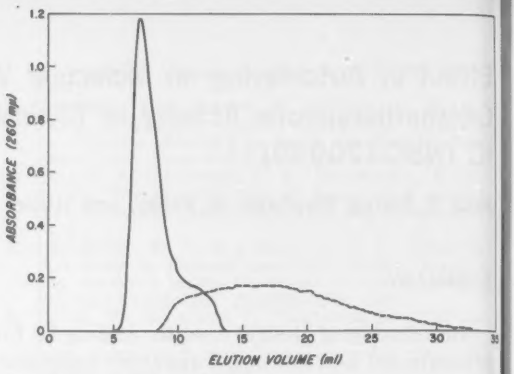

FIGURE 1.—Elution of two clinical dose formulations of poly IC from a column of Bio-Gel A150 m. Solid line: lot 12-99-003; broken line: lot 12-01-004A.

In every case, the elution profile of the autoclaved drug showed a slight trailing edge not visible with the unautoclaved lot. The extent of the difference of the two preparations was evident only upon chromatography on Bio-Gel A150 m (1% agarose; exclusion limit 1.5 $\times$ 10^6) (fig 1). Most of the unautoclaved poly IC was eluted as a sharp peak in what is probably the void volume, and the remainder (about 20%) appeared as a distinct secondary peak only slightly retained by the gel. On the other hand, autoclaved poly IC appeared dramatically different. No material appeared as early as the presumed void volume, and there was a broad distribution of polymer over a large elution volume. Repeated alternations of the two lots of polynucleotide on the same column yielded reproducible differences in elution pattern.

Despite this large difference in molecular

TABLE 1.—Chemotherapeutic effect of unautoclaved and autoclaved lots of poly IC against B_{16} melanomas in mice

Lot	Daily dose (mg/kg)	Survival time (days)	T/C (%)
Control	0	20.5	100
12-99-003	1.25	33.5	163
	2.5	34.5	168
	5.0	29.0	141
12-01-004A (autoclaved)	1.25	29.5	143
	2.5	30.0	146
	5.0	32.0	156

weight distribution, the two lots are indistinguishable in their activity against B_{16} melanoma in mice. Table 1 shows the results of an experiment in which mice were injected ip with either lot on Days 1–9 after tumor implantation. The T/C values for the autoclaved and unautoclaved dosage formulations are approximately the same.

DISCUSSION

It should be emphasized that although the relative molecular weight distributions are clearly shown, apparent molecular weights based on gel chromatography represent substantial overestimates because of the extreme asymmetry of the poly IC molecules. The true molecular weight range of these samples is probably substantially lower than the 5–15 $\times$ 10^7 suggested by the nominal exclusion limits of the agarose gels. To obtain precise molecular weights it would be necessary to combine these data with information on a parameter which was size-dependent in a different way, for instance, the sedimentation coefficient. In any case, however, it is clear that autoclaving leads to a substantial decrease in molecular weight and a broadening of the distribution.

It is of interest that Carleton et al report no denaturation of poly IC after autoclaving for 15 minutes at 20-pound pressure (11), despite the fact that, in our hands, there is considerable degradation. Considering that after 30 minutes they report measurable denaturation, it appears likely that these exposures are near a threshold value for the breakdown of poly IC and that small individual variations in procedure may lead to large differences in effect on structure and activity.

Precise molecular weight data would be valuable in comparing the results of different workers. Lampson et al (12) have reported a gradual loss of interferon-producing capacity as poly IC is degraded below 10^6, and it would be of interest to know whether the autoclaved material used in these studies had been degraded to this level. This appears improbable however. Lampson et al obtain good separations of their partially degraded material on a 4% agarose gel, roughly the equivalent of A15, from which our autoclaved preparation was totally excluded. Most likely, autoclaving decreased the molecular weight of poly IC samples, but insufficiently to affect biologic properties.

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Importance of Dose Schedules in Adjuvant Chemotherapy [1, 2, 3]

K. Karrar [4]

SUMMARY

Studies of systemic chemotherapy as an adjuvant to radical surgical treatment of bronchial carcinoma and carcinoma of the stomach have been conducted by the Viennese working group since 1955. This chemotherapy has avoided toxic symptoms and has not endangered the lives of patients or decreased the beneficial effect of surgical treatment in terms of survival time. Five studies with six different dose schedules are reported in 678 patients with bronchial carcinoma and 317 patients with stomach cancer. The cases of these patients are compared with those of control patients receiving surgical treatment only. In bronchial carcinoma, short-term treatment was ineffective (study No. 1). Long-term treatment (study No. 2), with eight intermittent courses given within 2 years of surgical treatment, showed an increased survival rate. Longer treatment (study No. 3), using 13 courses within 3 years, showed a further increase in survival rate. Patients with stomach cancer (study No. 4) treated with the same dose schedule used in study No. 2 showed no significant increase in survival rate and survival time, but a pilot study (study No. 5) using high pulse doses of cyclophosphamide (infusions of 25–30 mg/kg) gave encouraging results. It is important to note that patients in whom leukopenia developed because of adjuvant chemotherapy exhibited an increased survival rate compared to nonleukopenic patients treated by the same dose schedules. Because of the small number of patients treated in the study groups, the conclusions must be confirmed by further studies in larger cooperative groups.

[Cancer Chemother Rep 56:35–45, 1972]

Present methods of surgical treatment are, in general, so far advanced that they are unlikely to produce a further essential increase in the number of cured patients in the near future. However, in addition to the improvements afforded by early diagnosis, it will be possible to increase the number of cured patients, which is unsatisfactory today, by additional methods of combined treatment aimed at all cancer cells in the body.

Systemic cytostatic therapy is considered best for such general therapy, but at present there is no drug available which has a complete specific action against tumor cells, and chemotherapeutic effectiveness still depends on the adjustment of the relative toxic effects of known drugs on both tumor and host cells. Today, no other available methods are as effective as chemotherapy in producing tumor inhibition. For this reason, chemotherapy studies have been carried out in Vienna since 1955 in patients with bronchial carcinoma (1). Bron-

[1] Received Dec 17, 1970; revised June 16, 1971; accepted July 26, 1971.
[2] Presented at the 10th International Cancer Congress, Houston, Texas, May 25, 1970.
[3] Supported by the Austrian Research Council.
[4] Institute for Cancer Research, University of Vienna, Austria.

chial carcinoma was chosen for study for several reasons: (*a*) there is a particularly high incidence of the disease in Austria, (*b*) in Vienna, this type of tumor has been surgically treated only without additional specific tumor treatment, (*c*) the disease prognosis is poor and after radical surgical treatment more than half the patients die in the first 2 years as a result of recurrence or metastases, and (*d*) early recurrence permits a relatively rapid evaluation of the effectiveness of postoperative chemotherapy.

In general, private physicians in Austria refer suspected cases of bronchial carcinoma to university clinics for diagnosis and treatment. For this reason, surgeons in these clinics receive a relatively large number of early cases that can benefit from radical surgical treatment. At the beginning of this study, most of the patients with bronchial carcinoma were concentrated in the II University Clinic of Surgery so that special experience could be accumulated there.

METHODS

Study No. 1

In study No. 1, chemotherapy as an adjuvant to radical surgical treatment in patients with bronchial carcinoma consisted of one, two, or three courses of nitromin[5] beginning 5 or 6 days after surgical treatment and continuing within a period of 6 months. Each treatment course consisted of ten daily intravenous (iv) injections of 50–75 mg given at intervals of 6 weeks (2) (fig 1).

Study No. 2

Study No. 2 was undertaken in 1959 because the treatment in study No. 1 was ineffective in increasing the survival time and number of survivors (3,4). This second study used long-term treatment in three different dose schedules (fig 1; 2a, b, c). The drug protocol called for eight courses of drug treatment to be administered over a period of 2 years, each course of treatment consisting of either 50 mg of nitromin given daily for 10 days or 200 mg of cyclophosphamide[6] given daily for 30 days. The drugs were administered iv in hospitalized pa-

[5] Nitromin: NSC-10107; diethylamine, 2,2'-dichloro-*N*-methyl-, *N*-oxide, compd. with HCl (1:1).

[6] Cyclophosphamide: NSC-26271; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate; Cytoxan.

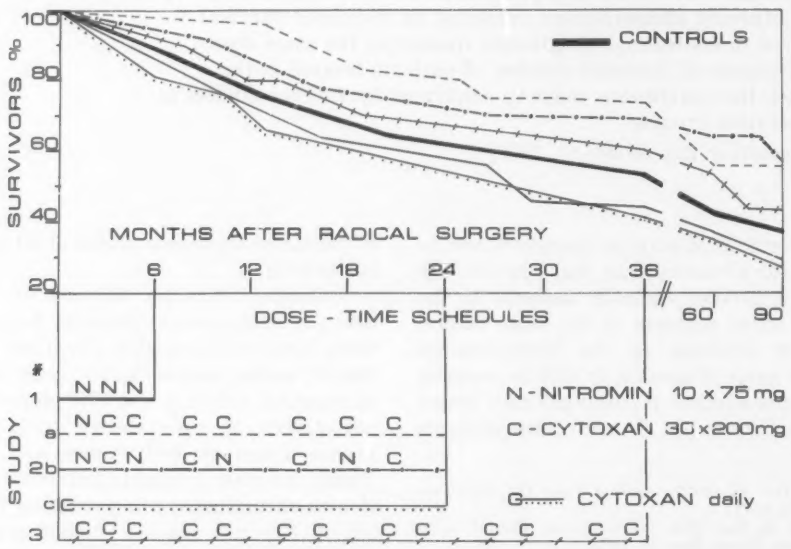

FIGURE 1.—Comparison of survival rates (life tables) of bronchial carcinoma patients (stage I) with and without adjuvant chemotherapy after surgical treatment.

tients and orally in outpatients (5,6). Because of severe toxicity, nitromin treatment was discontinued in 1962 and cyclophosphamide was used exclusively (7,8). The second study included patients who had received radical surgical treatment for stage I, II, and III bronchial carcinoma. These patients were selected for the study by microscopic pathological examination after surgical treatment, according to the international TNM classification system: stage I = T1 Na M0 and T2 Nc M0; stage II = T1 Nb M0, and T2 Nb M0; stage III = T1 Nc M0, T2 Nc M0, T3 Na M0, and T3 Nb M0.

The TNM classification system is recommended by the International Union Against Cancer (UICC) for international comparisons of the extent of disease. It describes the size of the primary tumor in category T (tumor), the involvement of regional lymph nodes in category N (nodes), and distant metastases in category M (metastases) (9,10). It has also been recommended to include the category of certainty (C) as the factor of evidence for categories T, M, and N (11,12).

Cyclophosphamide was chosen for use in this study because of its reported effectiveness in other clinical and experimental studies. This drug was also ideally suited for long-term cooperative studies because of its availability, stability, and ease of handling and administration. It is effective when administered either iv or orally (13–20).

Preliminary evaluations of this second study (2a, b, c) showed an improvement in survival time of patients receiving chemotherapy as an adjuvant to surgical treatment as compared to those receiving surgical treatment only (21–24).

Study No. 3

In 1962, because of the results of study No. 2, drug treatment was extended to 3 years after surgical treatment (study No. 3). In this study, cyclophosphamide was given at a dose of 200 mg/day for 30 days for a total dose of 6000 mg/course. Three such courses of treatment were given in the first 6 months and two courses in each of the five succeeding 6-month periods, for a total of 13 courses in 3 years.

All of the operations were performed by surgeons trained at the same surgical school, using the same type of operation, indication, and classification at either the II University Clinic of Surgery or at the First Surgery Department of the Vienna-Lainz Hospital. Each of these hospitals was divided into subdepartments with or without chemotherapy. The alternating grouping of patients was done according to the availability of beds in the subdepartment, but the surgical procedures and other medical care were independent of the location of the patient.

The evaluation of the effectiveness of the adjuvant chemotherapy was done through November 1, 1969, with life-table curves using the method of P. Sheehe (personal communication) and statistical calculations of survival rates according to Fisher's direct method (25).

Table 1 shows the number of patients with bronchial carcinoma treated in the three different studies. The total number of patients receiving radical operation and cytostatic treatment was 678 and they were divided according to disease stage using the international TNM classification system. The three simultaneous control groups of the three different studies showed no statistical differences and are there-

TABLE 1.—Number of bronchial carcinoma patients classified by disease stage and study No.

Disease stage	No. of patients in study No.—					Total treated	Untreated controls
	1	2a	2b	2c	3		
I	43	25	28	37	162	295	359
II	65	15	15	31	66	192	192
III	52	15	13	36	75	191	173
Total	160	55	56	104	303	678	724

fore combined in the overall results demonstrated in figure 1.

Study No. 4

A study with adjuvant chemotherapy (study No. 4) has been carried out since 1959 in 317 patients with stomach cancer, using the same dose schedules used in study No. 2.

Study No. 5

Because the statistical evaluation of study No. 4 showed no significant differences in the survival rates, a pilot study (study No. 5) has been started, using high-dose intermittent chemotherapy with protective autologous bone marrow transfusions (26,27). This therapy consisted of 2–3 iv infusions of 20–25 mg/kg of cyclophosphamide given at intervals of 3–5 days starting 3–6 days after the operation, and was continued for 3 years in courses of 400 mg of cyclophosphamide given iv for 10 days at intervals of 4–6 weeks. To be prepared for possibly severe and dangerous leukopenia caused by this high-dose chemotherapy, bone marrow was taken from the iliac crest of the patient and kept at –80 C for reinfusion.

RESULTS

The life-table curves for the patients with bronchial carcinoma (stage I) who received postoperative chemotherapy on different dose schedules are shown in figure 1. The graphically different life-table curves at the upper part of the figure correspond to the lines of the different dose schedules at the lower part. The life-table curves and the key for the dose-time schedules refer to the same scale on the abscissa indicating months after radical surgical treatment.

As can be seen in figure 1, the use of two dose schedules, continuous daily treatment with cyclophosphamide (No. 2c) and short-term treatment with nitromin (No. 1), lowers the survival rate compared with the control patients. The three intermittent long-term treatments increased the survival rates.

The statistical calculations based on the life-table curves in figure 1 show a significant difference between the two upper curves (2a, 2b) and the two lowest curves (1, 2c), with $z = 3.11$, and between the three upper curves (2a, 2b, 3) and the two lowest curves, with $z = 3.08$. The significance of the two upper curves compared with the untreated controls (surgical treatment only) is just on the borderline ($z = 2.7$).

Figure 2 shows some of the results of study No. 2 (2a, 2b, 2c). The life-table includes 57 treated patients in stage I and 89 patients in stage II after observation for 90 months or more, compared with 244 patients in stage I

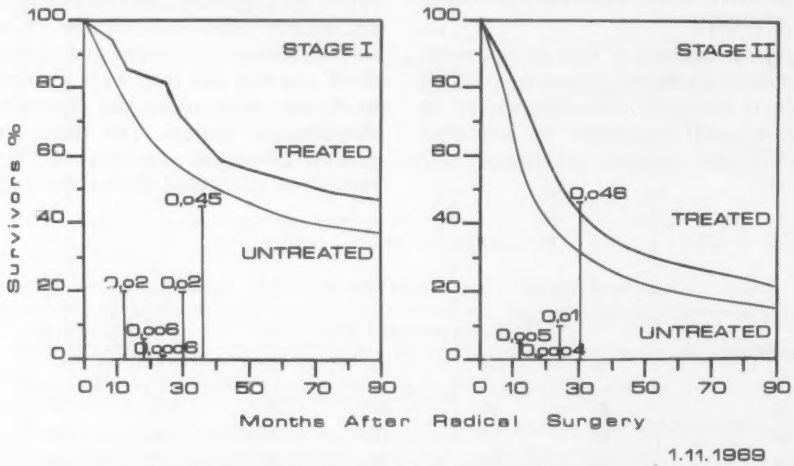

FIGURE 2.—Comparison of survival rates (life tables) of stage I and II bronchial carcinoma patients with and without adjuvant chemotherapy after surgical treatment.

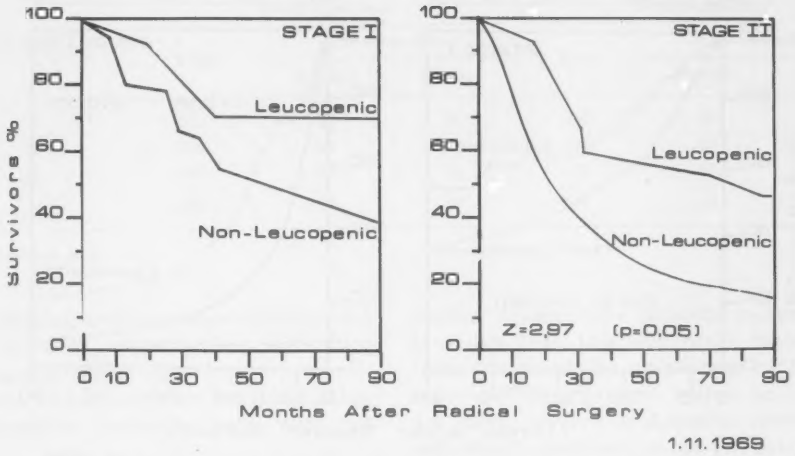

FIGURE 3.—Survival rates of leukopenic and nonleukopenic patients with stage I and II bronchial carcinoma after chemotherapy.

and 120 patients in stage II receiving surgical treatment only. All of the patients were dismissed from the hospital and postoperative mortality figures were excluded. The P values given in the figure, calculated using Fisher's direct method, indicate the significance of the increase in survival rate after postoperative chemotherapy in stage I and stage II patients for up to 3 and 2½ years respectively.

The drug-treated patients in figure 2 were further divided into two groups: those in whom leukopenia developed (<3000 cells/mm³), including 14 patients with stage I and 15 patients with stage II disease, and those in whom leukopenia did not develop after the same treatment. Their life tables are shown in figure 3.

As can be seen in figure 3, the survival rate is greater in leukopenic patients. The statistical calculation shows the significance ($z = 2.97$, $P = 0.05$) of the increased survival rate in leukopenic patients in stage II compared with nonleukopenic patients receiving the same treatment.

A further statistical study was made of pooled data from all the patients in study Nos. 2 and 3 who were operated on for bronchial carcinoma before November 1968. The comparison of all these patients treated with different long-term dose schedules showed no significant difference in survival rate, but dividing these patients receiving adjuvant chemotherapy into two groups, those with leukopenia (<3000 cells/mm³) and those without leukopenia, showed a significant difference in favor of the leukopenic patients as demonstrated in figure 4.

The numbers of patients in observation after surgical treatment are given at the top of figure 4 according to the time schedule in months. The first row shows leukopenic patients and the second row shows nonleukopenic patients, all of whom had received the same treatment. The statistical calculation shows the significance of the increased survival rate of the leukopenic patients in stage III ($z = 2.85$). In stage I the significance ($z = 2.61$) is just at the borderline (limit for $P = 0.05$, $z = 2.7$).

As in figure 2, the P values in figure 5 indicate the significance of the difference in survival rate in patients receiving long-term treatment compared with control patients receiving surgical treatment and surviving at least 2 years. The number of patients studied is given at the top of the figure.

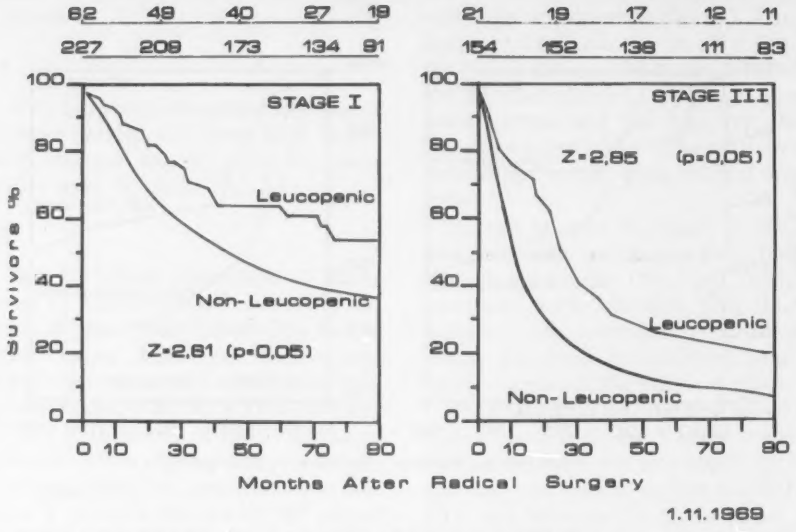

FIGURE 4.—Survival rates of leukopenic and nonleukopenic patients with stage I and III bronchial carcinoma after chemotherapy. Numbers of patients in observation after surgical treatment are given at top according to the time schedule in months. The first row shows leukopenic patients and the second nonleukopenic patients.

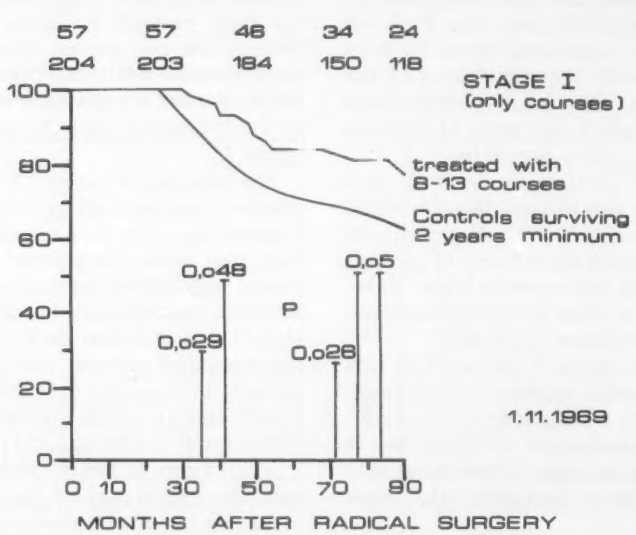

FIGURE 5.—Comparison of survival rates for stage I bronchial carcinoma patients who received 8–13 courses of chemotherapy after surgical treatment and patients who received surgical treatment only and survived at least 2 years. The first row shows treated patients and the second shows controls.

TABLE 2.—Number and percent of patients with wound-healing difficulties (per secundam)

Disease stage	Treated patients			Control patients*		
	Total	Per secundam		Total	Per secundam	
		Number	Percent		Number	Percent
I	295	52	18	359	78	22
II	192	36	19	192	45	23
III	191	26	14	173	47	27
	678	114	17	724	170	23

*Surgical treatment only.

In these studies, adjuvant chemotherapy did not result in more wound-healing difficulties than are usually observed in bronchial carcinoma patients as demonstrated in table 2. Also, the postoperative mortality rate was not increased.

After preliminary evaluations showing that doses causing leukopenia lead to an improved prognosis, a pilot study was conducted with a small group of patients given high doses of cyclophosphamide. Within 31 months after radical surgical treatment for bronchial carcinoma, these patients were treated with 3–6 iv infusions of 15–25 mg/kg of cyclophosphamide and intermittent courses of 400 mg every 10 days, with a 6–10-week interval without therapy. Thus far, all of the patients are still alive after 24–60 months of observation (average, 36 months). In contrast, of the untreated control patients (surgical treatment only), 46% had died by 36 months after the operation, and of a group of patients treated with 200 mg of cyclophosphamide per day, 34% had died by the same time.

The evaluation of 317 patients with stomach cancer (stage I, 135 patients; stage II, 97 patients; stage III, 85 patients) (study No. 4) receiving treatment identical to that used in study No. 2, did not show any statistically significant difference in the survival rate and survival time compared with 503 control patients receiving surgical treatment only (stage I, 192 patients; stage II, 145 patients; stage III, 166 patients).

The high-dose therapy used in the pilot study (study No. 5) caused leukopenia on an average of 14 days after the first cyclophosphamide infusion. Seventy-four patients recovered spontaneously from this leukopenia, increasing the leukocyte counts by an average of 1665 cells/mm^3 to 3728 cells/mm^3 within an average of 4.4 days. Severe leukopenia (average, 633 cells/mm^3) developed in six patients requiring bone marrow reinfusion. In an average of 7.3 days after bone marrow reinfusion, these patients recovered and the leukocyte counts increased to an average of 3692 cells/mm^3. With respect to the lowest level, the increase was 124% in patients who recovered spontaneously and 483% in those with reinfusion of bone marrow.

Thirty of the 55 patients in this pilot study are classified as stage III (T1 Nc M0, T2 Nc M0, T3 Na M0; T3 Nb M0). Thirteen of these patients were treated according to the schedule and 17 received only one or two infusions in the hospital and no further treatment thereafter. The evaluation of the effectiveness of this treatment by a comparison of the life tables of these treated stage III patients receiving radical operation as of February 1, 1970, with the life tables of stage III patients receiving radical operation only, is demonstrated in figure 6.

The numbers at the top of the figure show how many patients were being observed for the number of months indicated on the abscissa. The percent of survivors is given on the ordinate with regard to the operated patients dismissed from the hospital. The curves represent the 13 patients who followed their dose schedules, the 17 other patients who were given the first infusion only (20–25 mg/kg) with no cyclophosphamide treatment for 10 days, and the patients receiving surgical treatment only.

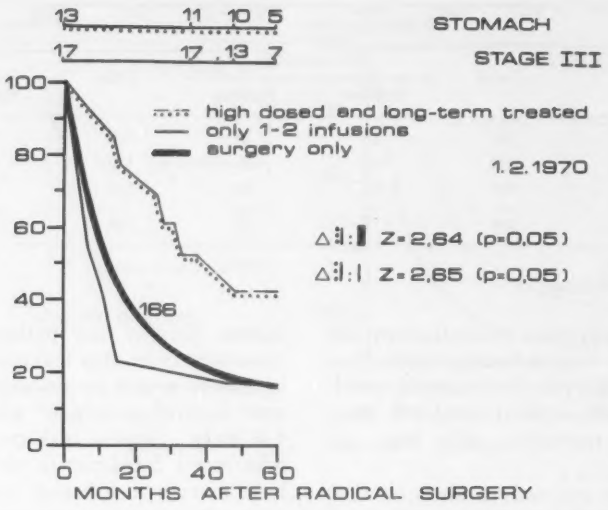

FIGURE 6.—Comparison of life tables for stage III stomach cancer patients receiving radical surgical treatment plus high-dose long-term treatment, surgical treatment plus 1–2 infusions (high-dose), and surgical treatment only.

In some cases the reason for discontinuing the treatment in the second group was the poor condition of the patient.

By statistical calculation the value $z = 2.65$ is just below the limit of significance (if $P = 0.05$, z should be 2.7). From these values the extent of the difference between the life tables of the patients receiving high-dose long-term cytostatic treatment and the life tables of the control patients and the patients treated for a short time only, can be seen. As a result, the continuation of this type of additional intermittent high-dose cytostatic therapy in stomach cancer seems to be successful and further application of combined therapy appears to be justified.

DISCUSSION

A number of clinical studies have shown that "short-term" adjuvant chemotherapy after radical surgical treatment has no beneficial effect on the course of the disease (28–32). The reason for this lack of effect seems to be that the tumor cells responsible for later recurrence and metastasis are already established at the time of surgical treatment in the form of micrometastases, or as the so-called dormant tumor cells. It is apparent that we have been unable to eradicate these tumors with drugs presently available. Treatment of these patients should therefore be directed at the prevention of future outgrowth of these micrometastases. The aim of this adjuvant chemotherapy should be to maintain a hostile environment for tumor growth with tolerable toxicity to the patient. This latter aspect is most important, because severe toxicity certainly interferes with any possible contribution of host factors to tumor inhibition. Perhaps the most favorable compromise between the desired tumor inhibition and the undesired immunosuppressive effects of cytostatic drugs, is intermittent high-dose pulse chemotherapy with adequate intervals between courses to allow recovery from toxic effects.

In adjuvant chemotherapy "longer-term" treatment may be necessary, and the questions of the most appropriate drug, dose level, and dose schedule have not been solved at the present time. One of the most problematic decisions

for a clinician to make in treating patients is the selection of the dose level of the chemotherapeutic agent to be used. The study presented here suggests that the dose level of a drug which produces overt signs of toxicity in a particular patient is the level most likely to achieve a prolongation of lifespan.

According to our findings it seems reasonable that the occurrence of leukopenia could be an indication that the nonleukopenic patient received a dosage which was below his individual limit of effectiveness. In addition to leukopenia, the duration of treatment is also very important, as has previously been reported (33,34). The first short-term dose schedule with nitromin was ineffective as was the continuous daily treatment with cyclophosphamide.

It is also suggested that the occurrence of mild leukopenia for a short time may represent a readily measurable manifestation of the chemotherapeutic potential of a drug in a given patient. We are not suggesting that the production of mild leukopenia will necessarily result in antitumor activity, but rather that it indicates that a pharmacologically active and tolerable dosage has been given to a particular patient. If the dose level is below that required to produce this type of minimal, but significant, toxicity, the clinician may not be certain that the dose level used was capable of eliciting any pharmacologic response (35–50).

The results of study No. 2, presented in figure 2, seem to contradict some results of the pooled data of study Nos. 2 and 3. The reason for this could be that the observance of the dose schedules for the first group of patients in study No. 2, who have been under observation for 90 months and more, was closer to the protocol than in the patients treated later when there was an increasing lack of treatment. The involvement of a larger number of private physicians with a different attitude toward the study of long-term treatment becomes a matter of critical importance.

Animal experiments with adequate tumor models which have been carried out in connection with the question of optimal dosage have shown that two or three high doses given in relatively short intervals were clearly superior to high single doses (51). The simultaneous administration of several drugs with different mechanisms of effect could prevent the resistance of the tumor; it could also destroy a higher percentage of tumor cells at the same time and distribute the unpleasant side effects of the therapy over different normal tissues or organs (52–62).[7] This principle has already proved successful in other types of cancer, particularly in leukemia, Wilms' tumor, and other solid tumors in advanced stages, and it seems to be logical that it would be even more effective in smaller tumors (63–69).

Our studies with different dose schedules and in patients with different tumors seem to show that long-term therapy is superior to short-term adjuvant therapy; intermittent courses are preferable to continuous daily treatment, and the high-dose therapy which shows individual effectiveness is preferable. Different tumors show different sensitivities to chemotherapy. Because of the small number of patients and the retrospective nature of this study our conclusions must be confirmed by further prospective studies in large cooperative groups. This need is further indicated because of similar results of other investigators (70–78).

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Effect of Two New Sulfonic Acid Esters of Aminoglycols on Chronic Myelogenous Leukemia [1]

Masami Hirano, Moriji Miura, Hisami Kakizawa, Akimitsu Morita, Tadaaki Uetani, Ryuzo Ohno, Kohei Kawashima, Hiroshi Nishiwaki, and Kazumasa Yamada [2]

In a screening study of the antitumor activity of a series of methanesulfonic acid esters of aminoglycols, Sakurai and El-Merzabani found two substances which have a potent antitumor effect on various experimental tumors, 3,3′-(methylimino)di-1-propanol, dimethanesulfonate, hydrochloride (NSC-84641) and 3,3′-iminodi-1-propanol, dimethanesulfonate (ester), hydrochloride (NSC-102627) (1,2). These compounds have the same alkylating group (methanesulfonyl) as busulfan (1,4-butanediol, dimethanesulfonate; NSC-750) (fig 1). Unlike busulfan, however, these two substances are water soluble. Their antitumor activity is unique in that they are effective not only in tumors sensitive to nitrogen mustard and its derivatives but also in those that are resistant, namely AH-7974, AH-66, and SN-36 leukemia (2,3).[3] They are also active against Jensen sarcoma, Walker 256 carcinosarcoma, Ehrlich carcinoma (2, see p 578), and L1210 leukemia (4).

We have been conducting a phase I study of these agents in the treatment of hematologic cancer and this paper reports the results obtained in chronic myelogenous leukemia.

MATERIALS AND METHODS

A total of 15 patients (5 men and 10 women) with chronic myelogenous leukemia have been treated with NSC-84641 (table 1). Their ages range from 20 to 69 years. Three patients had been previously treated with other agents, two with busulfan (patients 10 and 11) and one with NSC-102627 (patient 14). Patient 10 was in relapse despite daily administration of 6 mg of bulsulfan, and patient 11 had been treated at another hospital with busulfan at a dose of 2 mg daily for the preceding 7 weeks without any significant change in peripheral leukocyte counts or in the size of the spleen. Patient 10 could well be considered resistant to busulfan but the response to busulfan of patient 11 could not be evaluated because an insufficient dose was administered. Patient 14, who is also patient 23 (table 2), had been treated with NSC-102627 with a good response as defined below. The other 12 patients had received no prior treatment. In most cases a dose of 25 mg (approximately 0.5 mg/kg) of NSC-84641 dissolved in physiologic saline was given intravenously (iv). The doses were occasionally reduced by half or doubled. NSC-102627 was given iv daily at a dose of 50 mg (approximately 1 mg/kg) in 13 treatment courses to nine patients, four men and five women, 27–48 years old (table 2). Patient 19 had been treated with mitomycin C (NSC-26980) and then with busulfan, with complete remission obtained on each occasion. NSC-102627 was administered after relapse from a remission of 2.5 years' duration which was induced by busulfan. Patient 24 had received irradiation to the spleen in another hospital with satisfactory but transient improvement lasting only 1 month. Patients 16, 17, and 18 had complete remissions after treatment with NSC-84641 before the NSC-102627 administration.

Initial patients were hospitalized during the period of treatment for close observation; however, the absence of immediate toxic effects enabled us to treat others in the out-patient clinic.

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[3] Nitrogen mustard: NSC-762; diethylamine, 2,2′-dichloro-*N*-methyl-, hydrochloride.

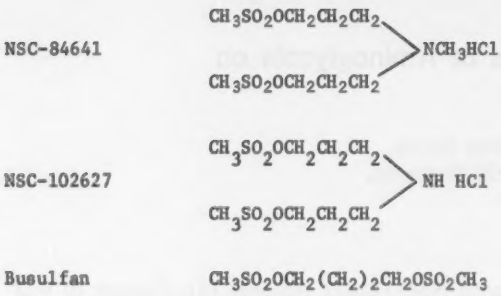

FIGURE 1.—Chemical structures of NSC-84641, NSC-102627, and busulfan.

NSC-84641 and NSC-102627 were discontinued when the leukocyte count decreased to a level of 50,000/mm³. Even if the reduction in leukocyte counts did not occur during drug administration, the treatment was stopped in most cases after a total dose of approximately 10 mg/kg of NSC-84641 and 20 mg/kg of NSC-102627 had been given. No maintenance therapy was given because of the iv route of administration of these agents.

Response was defined as follows: an excellent remission indicates a complete disappearance of palpable splenomegaly and a reduction in the leukocyte count to the normal range; a good remission means pronounced but not complete disappearance of splenomegaly with nearly normal leukocyte counts. A reduction of less than 50% in the size of the spleen is defined as a slight response.

Liver and kidney functions such as serum bilirubin, serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), serum protein, blood urea nitrogen (BUN), and serum creatinine were examined every 1–3 weeks.

RESULTS

NSC-84641

The total dose of NSC-84641 administered per patient was 135–1000 mg (median, 500 mg) and in most of the patients the dose given was between 8 and 10 mg/kg body weight. The duration of treatment ranged from 7 to 23 days (median, 16 days).

The effect of NSC-84641 on peripheral leukocytes is illustrated in figure 2. The start of the decrease in the leukocyte count varied but in

TABLE 1.—Effect of NSC-84641 on chronic myelogenous leukemia

Patient No.	Sex, age	Prior therapy	Dose (mg/day)	No. of treatments	Total dose (mg)	Wbc × 10³				Effect on spleen*	Length of remission (mos)	Overall clinical effect
						Before therapy	End of therapy	Nadir	End of therapy to nadir (days)			
1	F, 24	None	25, 50	22	1000	386	88	2.3	23	++	2	Good
2	F, 39	None	50	13	650	135	28	0.4	20	+++	12	Excellent
3	F, 27	None	50	8	400	252	250	2.2	37	+++	8	Excellent
4	M, 35	None	25	20	500	202	81	5.1	22	++	?	Good
5	M, 37	None	25	16	400	202	116	2.1	44	+++	>23	Excellent
6	F, 69	None	25	9	225	197	51	5.9	14	++	2	Good
7	F, 61	None	25	18	450	174	65	3.8	30	++	4	Good
8	F, 30	None	25, 50	23	900	131	49	1.1	23	+++	5	Excellent
9	F, 51	None	25	14	350	73	36	5.0	10	+++	>20	Excellent
10	M, 21	Busulfan	12.5, 50	22	575	61	9.4	3.0	20	++	2	Good
11	F, 25	Busulfan	25, 50	20	500	120	25	1.8	28	++	3	Good
12	F, 55	None	25	7	135	133	80	11.7	42	+++	2	Excellent
13	F, 20	None	25	20	500	176	173	0.9	40	+++	>3	Excellent
14	M, 28	NSC-102627	50	13	650	192	20	1.9	26	+++	2	Excellent
15	M, 44	None	25	8	200	120	67	3.6	30	+++	>1	Excellent

*+++ = Complete disappearance; ++ = pronounced shrinkage (>50%); + = shrinkage <50%.

TABLE 2.—Effect of NSC-102627 on chronic myelogenous leukemia

Patient No.	Sex, age	Prior therapy	Dose (mg/day)	No. of treatments	Total dose (mg)	Wbc × 10^3				Effect on spleen*	Length of remission (mos)	Overall clinical effect
						Before therapy	End of therapy	Nadir	End of therapy to nadir (days)			
16	F, 27	None	50	18	900	223	41	10.4	30	+++	2	Excellent
		NSC-84641	50	24	1200	158	11	7.3	12	+++	4	Excellent
17	M, 35	NSC-84641	50	19	950	141	51	?	?	++	?	Good
18	F, 24	NSC-84641	50, 100	18	1250	118	7.4	4.5	9	+	—	Fair
19	M, 37	Mitomycin C, busulfan	50	34	1700	875	26	15.2	7	++	2	Good
		Mitomycin C, busulfan, NSC-102627	25,50	30	1425	110	13	6.6	27	++	3.5	Good
		Mitomycin C, busulfan, NSC-102627	50	17	850	54	10	9.7	15	++	3	Good
20	F, 47	None	50	18	900	148	41	25.4	9	++	2	Good
21	F, 48	None	50	18	900	91	33	11.7	39	+++	3	Excellent
			50	24	1200	368	73	52.0	7	+	—	Slight
22	M, 28	None	50	22	1200	90	13	12.7	15	++	2	Good
23	M, 27	None	50	24	1200	538	46	29.6	7	++	—	Good
24	F, 27	Radiation	50	16	800	118	31	14.5	3	+++	1	Good

*+++ = Complete disappearance; ++ = pronounced shrinkage (>50%); + = shrinkage <50%.

many cases it was observed 7–10 days after the start of treatment. The rate of the decrease also varied in different cases; however, it was characteristic that the leukocyte count continued to fall even after the drug was discontinued. In patients 3 and 13 the leukocyte count did not change throughout drug administration, but it decreased sharply after the end of treatment.

The median leukocyte count at the start of treatment was 174,000/mm^3 (61,000–386,000/mm^3) and at the end of treatment was 65,000/mm^3 (9400–250,000/mm^3). Decreases in the leukocyte counts persisted after the end of treatment, reaching a median nadir of 2300/mm^3 (400–11,700/mm^3) in 26 days (10–44 days). Thus, myelosuppression due to NSC-84641 in the posttreatment period was severe and long-lasting.

The effect of NSC-84641 on splenomegaly was also remarkable. In nine patients complete disappearance of splenomegaly was noted and in another five the spleen decreased in size to less than 3 cm below the left costal margin. This shrinkage of the spleen paralleled the decrease in the leukocyte counts. Thus, an excellent remission characterized by normal leukocyte counts and a complete disappearance of palpable splenomegaly was induced in nine patients. In the other six patients a good remission was also obtained with almost normal leukocyte counts and significant shrinkage of the spleen. The median duration of unmaintained remission was 4.5 months.[4] It is noteworthy that remissions of more than 23 and 20 months' duration are still in progress in two patients (Nos. 5 and 9).

NSC-102627

This agent was given to nine patients at a total dose ranging from 800 to 1700 mg per patient (median, 1100 mg) for 16–34 days (median, 19 days). The doses of NSC-102627 were approximately twice as large as those of NSC-84641, that is, in most of the patients the dose was 18–20 mg/kg.

An excellent remission without clinically detectable splenomegaly was induced in three

[4] In this study relapse was defined as the time when the leukocyte counts increased to 50,000/mm^3.

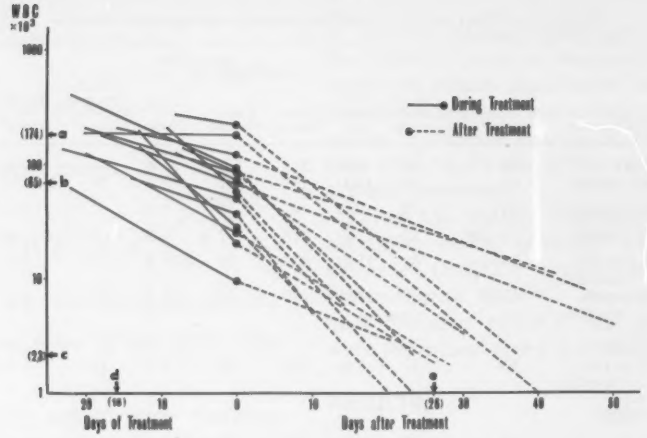

FIGURE 2.—Changes in wbc from the start of NSC-84641 treatment until its nadir. Values in parentheses on the ordinate indicate medians of wbc at the beginning (*a*) and end (*b*) of treatment and at its nadir (*c*). Those on the abscissa are medians of duration of treatment (*d*) and time from the end of treatment to the nadir of wbc in days (*e*).

patients. Good remissions occurred in five patients.

Reduction in the leukocyte count during treatment with NSC-102627 was also pronounced, but it was less significant as drug administration was continued (fig 3). The median leukocyte count at the start of treatment was 133,000/mm³ (54,000–875,000/mm³) and at the end of treatment was 28,500/mm³ (7400–73,000/mm³). The nadir of the leukocyte count (12,200/mm³) was reached 10 days after the start of treatment.

Side Effects

The only clinically significant side effect observed was excessive myelosuppression due to an overdose of NSC-84641. In three patients (Nos. 1, 2, and 8) severe leukopenia and throm-

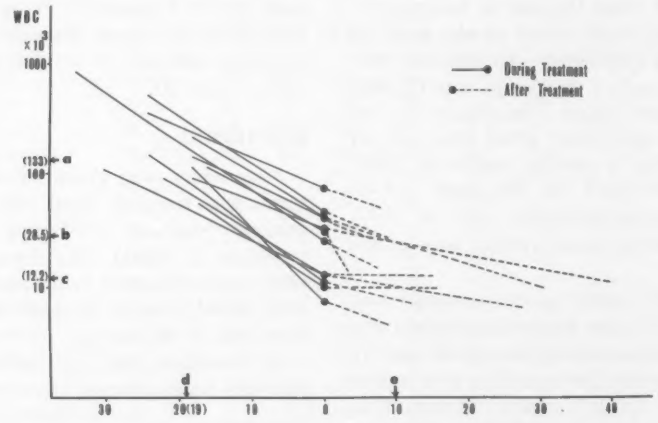

FIGURE 3.—Changes in wbc after NSC-102627 treatment. *See* Figure 2 for legend.

TABLE 3.—Effect of NSC-84641 and NSC-102627 on chronic myelogenous leukemia

Drug	Number of patients	Effective			Ineffective
		Excellent	Good	Slight	
NSC-84641	15	9	6	0	0
NSC-102627	9	3	5	1	0

bocytopenia occurred. Transfusions of fresh blood were required in patients 1 and 2. Gastrointestinal symptoms such as nausea and anorexia were not observed. There was no elevation in SGOT, SGPT, serum bilirubin, BUN, and creatinine levels at the end of treatment.

DISCUSSION

Results of therapy for chronic myelogenous leukemia with NSC-84641 and NSC-102627 are summarized in table 3. An excellent remission with complete disappearance of palpable splenomegaly was observed in nine of 15 patients treated with NSC-84641 and three of nine patients treated with NSC-102627. A good remission with pronounced but not complete disappearance of splenomegaly was induced in an additional six and five patients, treated with NSC-84641 and NSC-102627 respectively.

As stated previously, these two new alkylating agents, especially NSC-84641, have proved to be remarkably effective in the treatment of chronic myelogenous leukemia. They produce no toxic effects other than myelosuppression which resulted from an overdosage. Excessive myelosuppression was successfully prevented by discontinuing the drug after the total dose given to each patient reached 8–10 mg/kg (NSC-84641) and 18–20 mg/kg (NSC-102627) or after the leukocyte count decreased to approximately 50,000/mm³.

If the total dose of the drugs remained within these ranges, the myelosuppressive effect of the agents appeared to be selectively exerted upon the granulocytic series and thrombocytopenia was not observed. Anemia disappeared spontaneously as the disease was brought under control.

Results obtained with NSC-102627 are somewhat less satisfactory than those with NSC-84641. It is not the purpose of this paper to compare the effects of the two agents, but the lesser activity observed with NSC-102627 may well be due to the insufficient dosage. As seen in figure 3, the nadir of the leukocyte counts did not decrease further than 12,200/mm³ (median) with NSC-102627 as compared with 2600/mm³ in the case of NSC-84641. If a dose of NSC-102627 large enough to produce the same degree of reduction in the leukocyte count had been administered, comparable activity might have been obtained.

Does the effect of these agents surpass that of busulfan? It is interesting that NSC-84641 was quite effective in patient 10, who had relapsed during the administration of 6 mg/day of busulfan. This suggests that NSC-84641 might be effective in busulfan-resistant patients with chronic myelogenous leukemia. More studies, however, are needed for further evaluation.

In summary, the two new alkylating agents recently synthesized in Japan have proved to be valuable additions to the treatment of chronic myelogenous leukemia. The iv administration of these drugs has obviously been a great disadvantage. Recently, however, tablets of NSC-102627 in the form of tosylate became available and a preliminary report on the effect of oral administration in chronic myelogenous leukemia will follow.

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Combined Therapy with Cytosine Arabinoside (NSC-63878) and 1,3-Bis(2-chloroethyl)-1-nitrosourea (BCNU; NSC-409962) for Advanced Solid Tumors [1, 2, 3]

David W. Kinne [4, 5] and Edward W. Humphrey [4, 6]

SUMMARY

The effect of combination therapy with cytosine arabinoside and 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) was investigated in 23 patients with advanced cancer, 20 of whom could be evaluated. Only one objective response was noted. Cytosine arabinoside at a dose of 50 mg/m² intravenously in a 24-hour infusion, was given on Days 1, 5, 9, 13; 29, 33, 37, 41; and 57, 61, 65, 69 (three courses with 4-week intervals between initial injection of each course). The dose of BCNU varied (100 mg/m², 135 mg/m², or 160 mg/m²) and was administered in single injections on Days 1 and 57 (regimen A) or on Days 13 and 69 (regimen B). Reversible leukopenia and thrombocytopenia occurred more frequently with higher doses of BCNU. Nausea and vomiting were also frequent side effects. Further investigation of this drug combination and schedule is not indicated in the solid tumors studied.

[Cancer Chemother Rep 56:53–59, 1972]

In a continuing evaluation of new anticancer agents, the Veterans' Administration Lung Cancer Chemotherapy Group and Solid Tumor Group have used combined therapy with cytosine arabinoside and 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) in treating 23 patients with advanced cancer.

Cytosine arabinoside was first reported to have antitumor activity by Evans et al (1). The probable mechanism of action was inhibition of DNA synthesis by preventing the reduction of cytidine diphosphate to deoxycytidine diphosphate (2,3). Incorporation of cytosine arabinoside phosphate into RNA and DNA has also been shown (4). Its cytotoxic effects were expressed in the S phase of the cell cycle during DNA synthesis (5–7). BCNU, on the other hand, has properties of an alkylating agent, chiefly because of its chemical structure and its inhibitory effect against both dividing and nondividing L1210 leukemia cells in culture (8). BCNU is similar to nitrogen mustard[7] in the pattern of cell killing in the L1210 sys-

[1] Received Dec 21, 1970; revised Oct 4, 1971; accepted Nov 1, 1971.

[2] Cytosine arabinoside: cytosine, 1-β-D-arabinofuranosyl-, monohydrochloride; cytarabine; ara-C. Cytosine arabinoside and BCNU were supplied by Drug Research and Development, Chemotherapy, National Cancer Institute.

[3] These studies were conducted by the following investigators: *R. Dwight*, Boston, Mass; *J. Wolf*, Bronx, NY; *R. M. Whittington*, Cleveland, Ohio; *H. Majima*, East Orange, NJ; *D. T. Kuang*, Iowa City, Iowa; *F. Dietrich*, Memphis, Tenn; *C. Brindley*, Mountain Home, Tenn; and *H. P. Close*, Philadelphia, Pa.

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[7] Nitrogen mustard: NSC-762; diethylamine, 2,2'-dichloro-*N*-methyl-, hydrochloride.

tem (9) and has shown cross resistance with alkylating agents in certain animal tumors (10). However, there is further evidence that BCNU has a direct effect on de novo purine synthesis (9,11). A wide range of effectiveness against experimental animal neoplasms has been demonstrated with both BCNU (12) and cytosine arabinoside (13).

Skipper et al (5) described the optimum treatment regimen for BCNU in L1210 leukemia in mice as two thirds of the LD10 given as one dose. Against the same tumor, the optimum schedule of cytosine arabinoside was a dose every 3 hours for 24 hours on Days 2, 6, 10, and 14 after inoculation. The effectiveness of this schedule was attributed to the following observations: (*a*) the average doubling time of L1210 leukemia is 0.4–0.6 days, with the average S phase 90% of this time; (*b*) even at the maximum tolerated dose, the effective blood level of the drug persists for only 1 hour after infusion; and (*c*) shortly spaced doses over 24 hours affected the tumor during one or two average doubling times, with 3-day intervals between drug administration to allow for host recovery. Venditti et al (14) found that the optimum combination of BCNU and methotrexate[6] in the treatment of L1210 leukemia in mice is either: (*a*) BCNU at 0.35 of the optimum dose given alone on the first day of therapy only, plus methotrexate at 0.6 of the optimum dose given daily, or (*b*) BCNU at 0.5 of the optimum dose given on the first day of therapy only, plus methotrexate at 0.25 of the optimum dose given once every 4 days.

There has been recent interest in possible drug combinations acting synergistically against tumors (15). Schabel (16) noted therapeutically potentiating effects of BCNU and cytosine arabinoside against advanced L1210 leukemia, with cytosine arabinoside at the optimum solitary dose and BCNU at 0.5 of the optimum solitary dose, both given every 3 hours for 24 hours on Days 3, 7, 11, and 15 after inoculation. Mizuno and Humphrey (17) described additive but not synergistic effects of combination treatment with BCNU and cytosine arabinoside in mice bearing Sarcoma 180 (S180) or L1210 leukemia by measuring the uptake of H^3-thymidine by DNA in tumor tissue. To evaluate the possibility of synergistic action in man, a combined phase I and II study was undertaken using BCNU and cytosine arabinoside combination treatment in patients with advanced cancer.

DOSAGE SCHEDULE

BCNU was administered slowly intravenously (iv) within 10 minutes of reconstitution (each milliliter containing 3.3 mg of BCNU and 133.3 mg of mannitol in 10% ethanol), and any unused drug was discarded. Although flushing of the skin and local discomfort at the site of injection were frequently noted, these were minor problems. No phlebitis occurred. Cytosine arabinoside was administered in 1000 ml of 5% dextrose in water over a 24-hour period.

The dosage and schedule of administration are listed in table 1. Patients entered into the study were alternated between regimen A and regimen B, differing only in the timing of BCNU administration. This schedule of drug administration was selected to simulate the schedule which had proved effective in experimental animal tumor systems and yet would allow bone marrow toxicity to become apparent before the next scheduled course of drugs so that they could be withheld if necessary.

RESULTS

Of 23 cases entered into the study, 20 were evaluable. Three patients, all in regimen B, received only three injections of cytosine arabinoside and no BCNU. These patients died of progressive disease within a month after the

TABLE 1.—Dosage and schedule of administration of cytosine arabinoside and BCNU

Drug and dose	Regimen A (days of drug therapy)	Regimen B (days of drug therapy)
Cytosine arabinoside, 50 mg/m²/24 hrs iv in 1000 ml of 5% dextrose in water	1, 5, 9, 13, 29, 33, 37, 41, 57, 61, 65, 69	1, 5, 9, 13, 29, 33, 37, 41, 57, 61, 65, 69
BCNU, 100 mg/m² iv*	1, 57	13, 69

*100 mg/m² in 9 patients, 135 mg/m² in 9 patients, and 160 mg/m² in 2 patients.

[6] Methotrexate: NSC-740; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-.

last injection without significant drug toxicity. The 20 patients with evaluable cases included 12 in regimen A and eight in regimen B. As noted in table 2, these 20 patients can be divided into three groups. Eight patients received the full dose of therapy, ten received an intermediate dose, and two received a low dose of drugs. Eleven of 12 in these latter two groups were given no further drugs because of progressive disease while receiving therapy; in one case a patient in regimen B refused more cytosine arabinoside after the sixth dose (Day 33) because of severe nausea and vomiting with each injection. The average doses given to each of the three groups are listed in table 3. Possibly, the two patients who received the low dose of drugs were given an inadequate trial, and failure to show regression is due to this. However, in both cases rapid progression of disease during the month after the start of treatment precluded further therapy. The average doses of cytosine arabinoside and BCNU given to the 20 patients were 815 and 345 mg respectively.

Nineteen of 20 patients had no objective response (either stabilization or regression). These 19 patients had the following advanced cancers: adenocarcinoma of the colon, 7; adenocarcinoma of the stomach, 1; adenocarcinoma of the lung, 1; squamous cell carcinoma of the head and neck, 4; squamous cell carcinoma of the esophagus, 1; one each with pancreatic ampullary carcinoma, neurofibrosarcoma of the mediastinum, clear cell carcinoma, cylindroma of the pharynx, and an undifferentiated carcinoma in subcutaneous nodules.

One objective response occurred in a 36-year-old man who was admitted for treatment in April 1969 with bilateral pulmonary metastases 2 months after local excision of a left thigh rhabdomyosarcoma and simultaneous wedge resection of a pulmonary metastasis in the right upper lobe. Four weeks after completing a full course of combination therapy (regimen B: BCNU dose, 135 mg/m^2), all three metastatic lesions in the right lung had disappeared. The solitary left lung metastases, stable throughout this treatment, enlarged despite two subsequent

TABLE 2.—Full, intermediate, and low doses of drugs given to patients in regimen A and regimen B

Course	Drug (No. of injections received)	Regimen A		Regimen B	
		Total No. of patients	BCNU dose (mg/m^2)/No. of patients	Total No. of patients	BCNU dose (mg/m^2)/No. of patients
Full dose	Cytosine arabinoside (12) BCNU (2)	6	160/1 135/3 100/2	2	135/2
Intermediate dose	Cytosine arabinoside (8*) BCNU (1)	4	135/2 100/2	6	135/2 100/4
Low dose	Cytosine arabinoside (4) BCNU (1)	2	160/1 100/1	0	

*One patient received 7 injections, 1 patient 6 injections, and 2 patients 5 injections.

TABLE 3.—Average doses of drugs administered

Course	Regimen A average dose (mg)		Regimen B average dose (mg)	
	Cytosine arabinoside	BCNU	Cytosine arabinoside	BCNU
Full dose	1179	581	1328	536
Intermediate dose	591	195	597	195
Low dose	303	199	—	—
Average	838	389	781	280

courses of imidazole-4-carboxamide, 5-(3,3-dimethyl-1-triazeno)- (NSC-45388) and was resected in November 1969. He remained free of disease until May 1970, when a solitary right pulmonary metastases was noted. This lesion grew despite another course of BCNU and cytosine arabinoside (regimen B: BCNU dose, 135 mg/m²) and a wedge resection was done in October 1970. No other lesions were noted at thoracotomy, and his recovery was uneventful. He is an active outpatient, free of detectable recurrence as of July 1971.

TOXICITY

Reversible leukopenia occurred in 13 of 20 patients: the lowest white blood cell (wbc) count range was 2000–2900/mm³ in three patients, 3000–3900/mm³ in seven patients, and 4000–4900/mm³ in three patients. Leukopenia was more common in patients receiving higher doses of BCNU. Of the seven patients in whom no leukopenia developed, six received the lowest dose (100 mg/m²). As listed in table 4, eight of 12 patients in regimen A had leukopenia, as did

TABLE 4.—Hematologic and gastrointestinal toxicity correlated with dosage and schedule of drug administration

Course	BCNU dose (mg/m²)	*Regimen A* Wbc count Nadir (cells/mm³)	Wbc count Day noted	Wbc count Duration (days)*	Platelet count Nadir (cells/mm³)	Platelet count Day noted	Platelet count Duration (days)*	Nausea/ vomiting†
Full dose	160	2100	28‡	13	140	30	—	0
	135	3900	30	2	135	30	—	+
		3900	39	3	87	23	15	+
		3500	63§	4	151	27	—	+
	100	2600	18	7	69	67	10	+
		6100	58	—	92	55	18	0
Intermediate dose	135	2700	66	2	77	60	14	+
		3600	20	6	50	17	11	0
	100	5700	10	—	150	35	—	+
		9800	20	—	250	30	—	+
Low dose	160	4300	22	6	210	28	—	+
	100	5400	12	—	180	20	—	+

Course	BCNU dose (mg/m²)	*Regimen B* Wbc count Nadir (cells/mm³)	Wbc count Day noted	Wbc count Duration (days)	Platelet count Nadir (cells/mm³)	Platelet count Day noted	Platelet count Duration (days)	Nausea/ vomiting
Full dose	135	3500	30	2	70	34	7	+
		5200	38	—	80	41	10	+
Intermediate dose	135	3000	27¶	9	145	50	—	0
		4800	25**	7	72	62	13	+
	100	5300	45	—	50	45	21	+
		4400	6	9	78	40	15	+
		3900	31††	5	142	37	9	0
		6400	61	—	292	40	—	+

*Duration not listed if wbc count >5000/m³ or platelet count >100,000/m³.
†+ = Present, 0 = not present.
‡5th injection of cytosine arabinoside delayed 13 days; 2nd BCNU dose decreased to 135 mg/m².
§7th injection of cytosine arabinoside delayed 2 days.
¶ 5th injection of cytosine arabinoside delayed 9 days.
**5th injection of cytosine arabinoside delayed 7 days; patient refused more therapy after 6th injection due to nausea and vomiting.
††6th injection of cytosine arabinoside delayed 5 days.

five of eight patients in regimen B. Curiously, the leukopenia did not appear earlier in regimen A, occurring on Day 36 (average; range—Days 18–66) as compared to regimen B, where it developed on Day 24 (average; range—Days 6–31). Thus, the earlier administration of BCNU in regimen A was not associated with earlier development of bone marrow toxicity. The duration of leukopenia was short (range—2–13 days). Cytosine arabinoside administration was delayed in five patients from 2 to 13 days because of leukopenia. In one case in regimen B, drug administration was continued in spite of a wbc count of 4400 developing on Day 6, but this leukopenia was reversed by Day 15. The wbc count returned to normal in the remaining patients before the scheduled administration of drug, thus avoiding any delay. The only change in drug dose in the study was a decrease in BCNU from 160 to 135 mg/m² in a patient who had leukopenia for 13 days after having received the higher dose. Reversible thrombocytopenia, not associated with bleeding, was noted in ten of 20 patients. It occurred later than leukopenia (Day 44 on the average in regimens A and B) and lasted slightly longer (average, 14 days). In no case was administration of drug withheld because of thrombocytopenia. Nausea and vomiting were noted in 15 of 20 patients within a few hours of either BCNU or cytosine arabinoside administration, and usually subsided within 12 hours. This often became most severe in the later courses of cytosine arabinoside. Previously reported hepatic and renal toxicity (18) were not noted in these patients. In no case was death attributable to drug toxicity.

DISCUSSION

Both BCNU and cytosine arabinoside have proved effective against a variety of human neoplasms when given alone. DeVita et al (18) noted an objective response in 21% of 144 patients treated with BCNU. Regression occurred in patients with such diverse cancers as Hodgkin's disease, Ewing's sarcoma, malignant melanoma, and acute myelogenous leukemia. The effective dose was either 250 mg/m² in a single injection or 125 mg/m² in three daily doses. The dose-limiting toxic effects, leukopenia and thrombocytopenia, developed 3–4 weeks after drug administration and lasted 2–3 weeks. Reversible hepatic and renal toxic effects were noted in 26% and 10% of the patients respectively. Severe nausea and vomiting lasted 4–6 hours after drug infusion. Objective regression rates of 9.7% in patients with gastrointestinal adenocarcinoma (19) and 55% in patients with Hodgkin's disease (20) have also been reported. In addition, due to its ability to cross the blood-brain barrier, BCNU has been effective in meningeal leukemia (21) and recently in primary as well as metastatic brain tumors (22,33).

Like BCNU, cytosine arabinoside has caused regression of acute leukemia in children (24) and adults (25). In treating 89 patients with various cancers, Talley et al (26) observed a disappointing 11% objective response with cytosine arabinoside in solid tumors. Antitumor activity was more favorable in leukemia (four responses in four patients) and lymphosarcoma (15 responses in 23 patients). Bone marrow depression, the principal toxic effect, occurred 11–14 days after drug administration. Complete recovery was noted within 4 weeks.

The promising results of Schabel (16) described synergistic effects of combined BCNU-cytosine arabinoside treatment of L1210 leukemia, producing a high rate of cures even in advanced disease. Others have postulated additive or synergistic action (27) by combining a cycle-sensitive agent (such as cytosine arabinoside) with a cycle-insensitive agent (alkylating agent). Previous studies from this laboratory by Mizuno and Humphrey (17) demonstrated additive but not synergistic (or antagonistic) effects of combination treatment with cytosine arabinoside and BCNU. Measurement of H^3-thymidine uptake by DNA in S180 or L1210 leukemia in mice showed maximum inhibition of DNA-specific activity with BCNU given 24 hours before H^3-thymidine injection and with cytosine arabinoside given at the same time as H^3-thymidine. These times of maximum activity were unchanged when the drugs were given in combination. DNA-specific activity of hematopoietic tissues was compared with that of tumor tissue; neither drug showed selectivity on tumor when compared to spleen or lymph

node, although bone marrow was less affected. However, doses in the middle of the dose-response curve were utilized in this study. It was suggested that synergism might occur with increased doses.

In a recent study using combined cytosine arabinoside and BCNU therapy, three of 42 patients (one each with bronchogenic carcinoma, pancreatic carcinoma, and multiple myelomatosis) showed objective remission for longer than 1 month (28). A dose of 30–60 mg/m² of each agent was given iv every 4 days, with an average total dose of 350 mg of each drug given over a period of 14 days. No course lasted longer than 21 days, in anticipation of delayed toxicity. Bone marrow depression was the most important side effect in patients with solid tumors. Thus, on the average, more BCNU and less cytosine arabinoside was given over a shorter timespan in this study by van Eden et al than in ours, yet a disappointing regression rate was noted in both series.

In the present clinical study, the combination of BCNU and cytosine arabinoside in doses and schedules of administration approximating those producing maximal effect against experimental animal tumors, fails to show any synergistic action. Twenty cases were evaluated; an objective response in one patient (5%) with rhabdomyosarcoma may well have been caused by BCNU alone (18). Any synergism should be apparent even in a small series. Because of this disappointing response and the toxicity noted with higher doses of BCNU, further study of this dose combination and schedule seems unwarranted in the solid tumors studied.

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Patterns of Intravenous Glucose Tolerance and Insulin Response Before and After Treatment with Streptozotocin (NSC-85998) in Patients with Cancer [1, 2, 3]

Leonard Sadoff [4]

SUMMARY

Fifteen patients who had advanced cancer were given streptozotocin in doses ranging from 50 to 600 mg/kg. Comparable doses in rats have consistently caused severe insulin-dependent diabetes. Minor to moderate impairment of glucose tolerance developed in nine patients after treatment, but insulin was required in none. Three patients showed improved glucose tolerance after treatment, and three showed no change. An early common pattern of response (pattern I) which occurred in six patients consisted of a mild impairment of glucose tolerance associated with an increased insulin response, a pattern similar to one of abnormal carbohydrate metabolism reported in renal failure. Patients tested later tended to show a more normal glucose tolerance despite lower insulin responses (patterns II and III), indicating a possible hypoglycemic action of the drug. It is suggested that the effect of streptozotocin on glucose homeostasis in man is complex and is modified by the renal functional impairment and hepatic toxicity (hypoglycemic factor?) that accompanies the action of the drug.
[Cancer Chemother Rep 56:61–69, 1972]

The diabetogenic effect of streptozotocin has been repeatedly demonstrated in a variety of animal species including rodents, dogs, and monkeys (1–3). Its effect in rats is so consistent that it has been suggested as a more suitable drug than alloxan (NSC-7169) for inducing experimental diabetes in animal models (4). In contrast to animals, we have found that man is relatively resistant to the diabetogenic action of streptozotocin. Although the drug is effective in lowering serum insulin levels and causing regression of liver metastases in patients with islet cell carcinoma (5–7), the glucose plethora of uncontrolled diabetes has not been observed. Fifteen patients with advanced cancer received streptozotocin ranging from single doses of 50 mg/kg to cumulative doses of 600 mg/kg. Although minor aberrations of glucose tolerance occurred in some, insulin-dependent diabetes developed in none of the patients. In ten patients, abbreviated intravenous (iv) glucose tolerance, serum insulin, serum growth hormone, and phosphorus levels were measured. Five patients were studied in detail with full iv glucose tolerance, insulin response, growth

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hormone, and phosphorus curves measured before and after treatment.

PATIENTS AND METHODS

In June 1969, the Western Cooperative Cancer Chemotherapy Group began a phase I and II study of streptozotocin in patients with advanced cancer. Forty patients have been treated thus far without observing a single case of insulin-dependent diabetes. Fifteen patients were selected for the study of the effect of streptozotocin on carbohydrate metabolism. Patient data including age, weight, surface area, sex, diagnosis, and total dose are listed in table 1. Patients selected were in good nutrition with stable weights and had a negative family history for diabetes mellitus. Vitamin supplements including nicotinamide were withheld during the study period. Iv glucose tolerance tests were used rather than oral tests to eliminate the variable of incomplete absorption.

All patients received regular hospital diets (225 g carbohydrate) for 2 days before the study. (Table 2 shows that some patients lost considerable weight during the treatment period and could not entirely adhere to the diet. Since starvation is known to impair glucose tolerance, this factor must be taken into account in interpreting the findings in these patients.) Iv glucose tolerance tests were performed in the morning after an overnight fast. Baseline blood samples were drawn and part of the serum was immediately frozen for insulin determinations. A bolus of 0.5 g/kg of 50% glucose was then injected rapidly (2–4 minutes) into an anticubital vein. In screening the first ten patients, blood samples were drawn one-half hour, 1 hour, and 2 hours after glucose administration. Subsequently, five patients were studied in more detail with blood samples drawn at 5-minute, 15-minute, 30-minute, 45-minute, 1-hour, and 2-hour intervals after glucose administration. Blood glucose levels (normal, 70–105 mg/100 ml) and serum phosphorus levels (normal, 2.7–4.5 mg/100 ml) were measured by autoanalyzer. Insulin and growth hormone determinations were performed at Bio Science Laboratories, Van Nuys, California.

Streptozotocin was produced by the Ben Venue Laboratories, Bedford, Ohio. The contents of the vial were diluted with 18.6 ml water of injection and injected rapidly (2–4 minutes) directly into a vein. All patients were given 2 g/m^2 at weekly intervals for from 2 to 6 weeks. The drug was discontinued if no objective re-

TABLE 1.—Patient data

Patient No.	Age/sex	Weight (kg)	Surface area (m^2)	Diagnosis	Total dose [(g)/(mg/kg)]
1	75/M	77	1.9	Adenocarcinoma, pancreas	7.6/100
2	66/M	50	1.5	Adenocarcinoma, pancreas	9.0/180
3	62/M	60	1.7	Carcinoma, mouth	6.8/110
4	62/F	62	1.65	Carcinoma, lung	9.8/320
5	62/M	51	1.7	Carcinoma, lung	14.6/285
6	55/M	86	2.0	Carcinoma, lung	24/280
7	59/F	60	1.65	Adenocarcinoma, pancreas	9.9/165
8	68/M	57	1.7	Carcinoma, prostate	6.8/120
9*	54/F	51	1.5	Carcinoma, ovary	6.0/120
10	66/F	70	1.65	Synovial sarcoma	42/600
11	56/M	85	2.0	Carcinoma, parotid	12/140
12	70/M	50	1.5	Adenocarcinoma, pancreas	6.0/120
13	49/F	87	2.0	Synovial sarcoma	7.0/80
14	68/F	78	1.8	Carcinoma, ovary	9.6/125
15	62/M	69	1.85	Carcinoma, kidney	11.1/160

*Patient with maturity-onset diabetes mellitis.

sponse occurred by 6 weeks or if renal toxicity (azotemia) supervened. One patient who had an objective response (patient No. 10) was treated for 6+ months (*See* Results.)

RESULTS

Baseline measurements of carbohydrate metabolism were obtained in 15 patients before treatment. Table 2 shows that six of the 15 patients had pre-existing abnormalities of glucose tolerance before treatment, ie, fasting blood sugar or 2-hour blood sugar greater than 115 mg/100 ml (criteria established by Lozner et al [8]). Only one of these six patients was known to have clinically documented diabetes mellitus before this study (patient No. 9). The finding of decreased glucose tolerance in several of our patients was not unexpected since several investigators have reported abnormal glucose tolerance in patients with cancer (9–11).

After baseline studies, seven of 15 patients were arbitrarily studied on Day 10, 3 days after their second injection. Another patient (No. 3) who received two injections was not studied until Day 46 because he went into renal shutdown shortly after his second injection. The decision to study the largest group of patients after their second injection was based on the careful work of Junod et al (4), who showed that insulin-dependent diabetes could be consistently produced in male Wistar rats with a single or cumulative dose of 100 mg/kg of streptozotocin. A comparable dose in the patients studied was usually reached after their second injection. It became obvious however, as the study progressed, that the human response at this point was far different than the response reported in rodents.

The effect of streptozotocin on carbohydrate metabolism in 15 patients with cancer is shown in table 2. Decreased glucose tolerance developed in nine patients (Nos. 1, 2, 3, 8, 9, 11, 12, 13, and 14) while three patients (Nos. 2, 10, and 15) had improved glucose tolerance and three showed no essential change. The most common pattern (pattern I) was seen in those patients who were studied soon after their second injections (average cumulative dose, 100 mg/kg). In five of seven patients, there was decreased glucose tolerance associated with an increased insulin response and decreased phosphorus utilization (patient Nos. 1, 2, 8, 12, and 13). Growth hormone, when it was measured, showed no significant variation from baseline except in patient No. 12. In the five patients who were studied after three injections (average cumulative dose, 150 mg/kg), only one (No. 11) showed pattern I. Three patients had increased glucose tolerance with paradoxical decrease in insulin response (pattern II). Decreased glucose tolerance with decreased insulin response (pattern III) developed in the remaining patient (No. 14). The single patient studied after four injections (No. 5) showed no essential change. Two patients studied after six injections (average cumulative dose, 300 mg/kg) showed slightly decreased glucose tolerance with decreased insulin response (pattern III).

Four patients were studied twice to determine whether a temporal effect occurred. Pattern I developed in patient No. 2 after his second injection and pattern II developed after his third injection. Pattern II developed in patient No. 10 after her second injection and she was tested 6 months later after receiving 600 mg/kg and showed no significant change. Pattern I developed in patient No. 11 after his third injection and he was retested 1 month later and showed an apparent shift toward baseline values. Interestingly, growth hormone levels were significantly higher in this patient at this time.

GROWTH HORMONE

Although there was some variation in growth hormone levels after streptozotocin treatment, increased 1-hour levels developed in several patients, especially those tested after 3 weeks (Nos. 3, 4, 5, 6, 7, and 11).

RENAL TOXICITY

Renal toxicity occurs regularly with streptozotocin treatment and is the limiting factor in dosage (12). In the present series, azotemia (creatinine level $>$ 2 mg/100 ml) developed in seven of 15 patients (table 2). Decreased glucose tolerance developed in five of these azotemic patients while two showed improved

TABLE 2.—Effect of streptozotocin on metabolic values

Patient No.	Weight (kg)		Interval after glucose administration that blood sample was drawn (min)	Blood glucose (mg/100 ml)		Serum insulin (micro units/ml)		Serum phosphorus (mg/100 ml)		Serum growth hormone (ng/ml)		Day(s) tested after first injection
	Before treatment	After treatment		Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment	
1	77	73	0	93	138	10	27	3.6	2.6		—	Day 10 (2 injections; total dose, 100 mg/kg)
			30	267	306	22	49	3.3	2.5		—	
			60	228	282	24	36	3.1	2.4		—	
			120	170	245	22	37	2.7	2.3			
2	50	49	0	87	103, 61	5	8, 4	3.3	2.5, 1.7		—	Day 10, 120 mg/kg Day 56, 180 mg/kg
			30	162	210, 137	10	14, 4	3.0	2.3, 1.6		—	
			60	120	164, 95	13	18, 6	3.1	2.2, 1.6		—	
			120	82	105, 64	7	10, 5	3.2	2.2, 1.8		—	
3	60	51	0	55	117	14	6	3.8	6.3	2	2	Day 46 (2 injections; total dose, 100 mg/kg)
			30	235	252	44	17	3.1	6.3			
			60	157	197	68	19	2.9	6.2	3	9	
			120	87	134	23	14	2.8	6.1	2	2	
4	62	60	0	100	127	8	13	3.2	1.5	2	2	Day 38 (6 injections; total dose, 320 mg/kg)
			30	225	230	31	19	2.9	1.3			
			60	148	162	33	34	2.7	1.2	7	13	
			120	112	105	18	14	2.9	1.3	2	2	
5	51	51	0	105	103	7	5	3.3	2.2	5	8	Day 24 (4 injections; total dose, 285 mg/kg)
			30	168	170	8	7	2.9	1.9			
			60	138	140	8	8	2.8	1.7	14	38	
			120	115	110	4	9	3.0	1.8	2	5	
6	87	77	0	108	107, 105	4	6, 4	3.7	1.0, 3.9	2	2, 1	Day 38 (6 injections; total dose 280 mg/kg) 6 mos, 280 mg/kg
			30	238	238, 195	18	8, 13	3.1	0.9, 3.6			
			60	170	177, 160	15	9, 14	2.9	0.9, 3.5	3	5, 2	
			120	120	137, 100	5	5, 9	3.0	1.1, 3.6	4	3, 2	
7	60	48	0	100	105	11	5	4.0	3.7	2	2	Day 40 (3 injections; total dose, 165 mg/kg)
			30	224	227	36	14	3.5	3.5			
			60	197	184	48	16	3.3	3.1	2	7	
			120	122	114	15	11	3.2	3.0	4	3	
8	57	47	0	95	95	0	7	4.7	3.5	4	2	Day 10 (2 injections; total dose, 120 mg/kg)
			30	178	220	18	18	4.6	3.2			
			60	148	177	14	26	4.6	3.2	4	3	
			120	89	117	11	11	4.4	2.9	2	2	
9	51	49	0	117	177	6	9	3.8	2.0	6	2	Day 10 (2 injections; total dose, 120 mg/kg)
			30	238	309	21	6	3.6	3.0			
			60	168	257	19	15	3.5	2.6	13	4	
			120	135	222	15	10	3.7	2.5	2	2	
10	70	70	0	128	107, 122	15	6, 5	2.9	1.7, 2.7	2	2, 1.5	Day 10, 95 mg/kg 6 months, 600 mg/kg
			30	295	246, 265	36	49, 30	2.5	1.4, 2.0			
			60	242	167, 157	88	49, 44	2.4	1.5, 1.8	2	2, 0.5	
			120	107	83, 77	30	20, 18	2.3	1.5, 1.9	2	2, 1.5	
11	85	83	0	97	100, 96	16	12, 14	3.4	1.5, 2.5	>2	0.7, 1.0	Day 17 (3 injections; total dose, 140 mg/kg) Day 47
			5	318	328, 315	80	75, 72	3.3	1.4, 2.4	>2	0.7, 4.0	
			15	291	300, 290	56	68, 50	3.0	1.3, 2.0	>2	0.8, 4.0	
			30	227	292, 255	59	115, 59	2.7	1.1, 2.0	>2	0.9, 8.0	
			45	191	246, 215	58	90, 56	2.6	1.2, 2.1	>2	1.1, 9.0	
			60	165	213, 177	54	84, 56	2.6	1.3, 2.0	>2	1.7, 9.0	
			120	78	133, 100	23	56, 40	3.0	1.6, 2.1	>2	2.0, 1.0	

in 15 patients with advanced cancer

Serum creatinine or creatinine clearance		Electrolytes* (mEq/liter)			Liver function tests†			
Before treatment	After treatment		Before treatment	After treatment		Before treatment	After treatment	Response
0.9 mg/100 ml	3.8 mg/100 ml	CO_2	24	17	Bilirubin	0.3 mg/100 ml	—	0–0
					SGOT	37.0 units	—	
					LDH	46.0 units	—	
					Alk phos	45.0 IU	40.0 IU	
					Alb/Glob	3.3/3.3 g/100 ml		
0.95 mg/100 ml	2.0 mg/100 ml	CO_2	25	20	Bilirubin	2.2 mg/100 ml	0.5, 2.3 mg/100 ml	I–A (2 mos)‡
	1.6 mg/100 ml	Cl	100	105	SGOT	176.0 units	27.0, 214.0 units	
		K	4.3	4.4	LDH	32.0 units	35.0, 89.0 units	
					Alk phos	194.0 IU	154.0, 204.0 IU	
					Alb/Glob	2.4/3.7 g/100 ml		
0.85 mg/100 ml	10.0 mg/100 ml	CO_2	25	20	Bilirubin	0.5 mg/100 ml	—	I–A (1–2 mos)
		Cl	98	92	SGOT	22.0 units	32.0 units	
		K	4.2	3.4	LDH	36.0 units	65.0 units	
					Alk phos	12.0 IU	44.0 IU	
					Alb/Glob	4.1/3.2 g/100 ml	3.1/3.1g/100 ml	
0.7 mg/100 ml	1.05 mg/100 ml	CO_2	29	27	SGOT	36.0 units	37.0 units	0–0
		Cl	103	107	LDH	77.0 units	92.0 units	
		K	4.5	3.1	Alk phos	39.0 IU	36.0 IU	
					Alb/Glob	3.2/3.5 g/100 ml	3.1/3.1 g/100 ml	
0.95 mg/100 ml	0.9 mg/100 ml	CO_2	34	27	Bilirubin	0.2 mg/100 ml	—	0–0
		Cl	95	100	SGOT	51.0 units	20.0 units	
		K	4.1	4.1	LDH	87.0 units	78.0 units	
					Alk phos	48.0 IU	15.0 IU	
					Alb/Glob	1.8/3.6 g/100 ml	3.2/3.5 g/100 ml	
157 ml/min	53 ml/min	CO_2	27	20	SGOT	26.0 units	41.0 units	II–B
		Cl	106	108	LDH	48.0 units	70.0 units	
		K	4.9	3.8	Alk phos	29.0 IU	41.0 IU	
					Alb/Glob	3.5/2.5 g/100 ml	3.4/2.7 g/100 ml	
0.9 mg/100 ml	4.2 mg/100 ml	CO_2	29	19	SGOT	20.0 units	45.0 units	I–A (2 mos)
		Cl	103	118	LDH	62.0 units	115.0 units	
		K	4.3	3.0	Alk phos	30.0 IU	22.0 IU	
					Alb/Glob	3.8/3.5 g/100 ml	3.5/5.3 g/100 ml	
66 ml/min	45 ml/min	CO_2	26	—	SGOT	40.0 units	42.0 units	0–0
		Cl	100	—	LDH	50.0 units	78.0 units	
		K	4.2	—	Alk phos	32.0 IU	54.0 IU	
					Alb/Glob	3.6/3.0 g/100 ml	3.9/2.9 g/100 ml	
0.75 mg/100 ml	3.2 mg/100 ml	CO_2	26	16	SGOT	26.0 units	42.0 units	0–0
		Cl	98	110	LDH	53.0 units	240.0 units	
		K	4.1	2.4	Alb/Glob	3.1/3.9 g/100 ml	3.5/5.3 g/100 ml	
0.85 mg/100 ml	2.2 mg/100 ml	CO_2	25	24	SGOT	28.0 units	119.0 units	I–B (5 mos)
	2.5 mg/100 ml	Cl	97	112	LDH	77.0 units	116.0 units	
		K	4.2	3.0	Alk phos	28.0 IU	51.0 IU	
					Alb/Glob	3.6/3.1 g/100 ml	4.3/3.0 g/100 ml	
1.05 mg/100 ml	1.5 mg/100 ml	CO_2	24	27	SGOT	38.0 units	80.0 units	0–0
		Cl	98	100	LDH	32.0 units	48.0 units	
	1.4 mg/100 ml	K	4.2	3.2	Alk phos	22.0 IU	40.0 IU	
					Alb Glob	—	—	

Patient No.	Weight (kg)		Interval after glucose administration that blood sample was drawn (min)	Blood glucose (mg/100 ml)		Serum insulin (micro units/ml)		Serum phosphorus (mg/100 ml)		Serum growth hormone (ng/ml)		Day(s) tested after first injection
	Before treatment	After treatment		Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment	
12	50	50	0	97	105	11	17	3.8	3.0	0.6	4	Day 10 (2 injections; total dose, 120 mg/kg)
			5	282	296	73	85	3.7	2.9	0.6	3	
			15	237	277	25	37	3.6	2.9	0.7	4	
			30	207	205	20	28	3.4	2.9	0.8	10	
			45	192	177	22	30	3.4	3.0	0.8	9	
			60	167	155	16	28	3.5	3.0	0.5	6	
			120	110	135	16	20	3.3	3.0	5	6	
13	87	87	0	103	118	36	47	4	3.3	2	2	Day 10 (2 injections; total dose, 80 mg/kg)
			5	304	322	165	190	3.8	2.7	2	2	
			15	365	277	155	180	3.5	2.7	2	2	
			30	215	243	175	105	3.2	2.7	2	2	
			45	178	202	150	98	3.1	2.8	2	2	
			60	145	183	125	120	3.0	2.7	2	2	
			120	94	125	57	80	3.3	2.7	2	2	
14	79	68	0	97	102	6	6	3.5	4.7	2	2	Day 42 (3 injections; total dose, 125 mg/kg)
			5	220	290	49	39	3.4	4.6	2	2	
			15	197	270	38	22	3.4	4.6	2	2	
			30	150	227	21	17	3.2	4.6	2	2	
			45	138	184	17	16	3.3	4.5	2	2	
			60	120	148	15	15	3.3	4.6	2	2	
			120	100	137	6	14	3.3	4.4	3	2	
15	69	68	0	120	113	14	10	3.7	2.6	0.5	0.5	Day 27 (3 injections; total dose, 160 mg/kg)
			5	330	315	31	27	3.6	2.6	0.5	0.5	
			15	300	272	45	31	3.2	2.3	0.5	0.5	
			30	280	265	54	38	3.0	2.2	0.5	0.5	
			45	237	232	65	33	2.9	2.2	0.5	0.5	
			60	233	203	73	35	2.7	2.2	3.0	0.7	
			120	152	135	37	20	2.6	2.3	0.5	4.0	

* Electrolytes, normal values: CO_2—24–30 mEq/liter; Cl—98–106 mEq/liter; K—3.5–5.0 mEq/liter.
† Liver function tests, normal values: Bilirubin—1.0 mg/100 ml; Serum glutamic oxaloacetic transaminase (SGOT)—1–36 units; Lactic dehydrogenase (LDH)—24–78 units; Albumin (Alb)—3.5–5.5 g/100 ml; Globulin

glucose tolerance. Thus, five of the nine patients in whom decreased glucose tolerance developed were azotemic, and in the remaining four patients creatinine clearance was reduced in all but one.

Renal glycosuria and the adult Fanconi syndrome have been described after streptozotocin treatment (12). Glycosuria in the presence of normal fasting glycemia developed in most patients in this series. In five patients (Nos. 3, 4, 11, 12, and 15) 24-hour urine glucose determinations were measured after streptozotocin treatment, and in no patient did the value exceed 5 g/24 hours.

DISCUSSION

The diabetogenic action of streptozotocin has been shown to be dose dependent in animals. In rats, a dose of 25 mg/kg produces mild "chemical" diabetes while a dose of 100 mg/kg produces severe diabetes with ketonuria and insulin dependence. Further, the effect of two injections of 25 mg/kg 3 days apart was shown to have a cumulative effect producing severe diabetes (4). A dose of 50 mg/kg is frequently diabetogenic in monkeys but the disease is apparently somewhat responsive to tolbutamide given iv (3).

In the present study, all patients except one received at least 100 mg/kg and one patient received 600 mg/kg. Minor aberrations of glucose tolerance developed in nine patients but none required insulin. There was no correlation between total cumulative dose and the degree of glucose intolerance. No impairment of glucose tolerance developed in the patient who re-

in 15 patients with advanced cancer—Continued

Serum creatinine or creatinine clearance		Electrolytes* (mEq/liter)			Liver function tests†			
Before treatment	After treatment		Before treatment	After treatment		Before treatment	After treatment	Response
1.25 mg/100 ml	2.1 mg/100 ml	CO_2	25	—	Bilirubin	0.7 mg/100 ml	—	0–0
		Cl	102	—	SGOT	54.0 units	48.0 units	
					LDH	65.0 units	58.0 units	
					Alk phos	29.0 IU	42.0 IU	
					Alb/Glob	3.7/3.4 g/100 ml	3.2/3.0 g/100 ml	
0.8 mg/100 ml (110 ml/min)	0.75 mg/100 ml (118 ml/min)	CO_2	26	24	SGOT	24.0 units	32.0 units	0–0
		Cl	97	104	LDH	96.0 units	73.0 units	
		K	3.9	4.0	Alk phos	22.0 IU	33.0 IU	
					Alb/Glob	3.7/4.4 g/100 ml	—	
0.95 mg/100 ml (74 ml/min)	1.25 mg/100 ml (38 ml/min)	CO_2	—	—	SGOT	34.0 units	48.0 units	0–0
		Cl	—	—	LDH	54.0 units	58.0 units	
		K	—	—	Alk phos	45.0 IU	47.0 IU	
1.1 mg/100 ml	1.25 mg/100 ml	CO_2	26	28	SGOT	100.0 units	138.0 units	0–0
		Cl	91	100	LDH	95.0 units	180.0 units	
		K	3.7	4.3				

(Glob)—2.0–3.8 g/100 ml; Alkaline phosphatase (Alk phos)—9–36 International units.
‡ Patient responded briefly after second injection with normalization of liver function tests, but relapsed again after third injection.

ceived 600 mg/kg (No. 10) even after 6 months of treatment. (One might speculate whether a single massive dose of streptozotocin, ie, 1 g/kg, could produce insulin-dependent diabetes in man. It is likely that such a dose would cause fatal renal toxicity.)

The most common alteration of carbohydrate metabolism (pattern I) occurred in six of the 15 patients. In five of these, the abnormality was observed after their second injection, and in one patient it was observed after the third injection. This abnormality (decreased glucose tolerance and increased insulin response) has been reported to occur in prediabetes (13) as well as in renal failure (14). Azotemia developed in four of the seven patients with pattern I during the course of therapy.

RENAL FAILURE EFFECT?

The role of renal failure in the impaired glucose tolerance associated with streptozotocin therapy is worthy of consideration. Five of our nine patients in whom decreased glucose tolerance developed after streptozotocin treatment were azotemic. In the remaining four patients, creatinine clearance was reduced 25%–75%. Two patients with azotemia (Nos. 7 and 10) failed to have impaired glucose tolerance. (It has been shown that some uremic patients retain normal glucose tolerance [14]).

Renal functional impairment has not been investigated in animal studies with streptozotocin. Thus, the role of renal failure in drug-induced animal diabetes is unknown. Animal deaths attributed to diabetic acidosis might be due to associated renal failure. The urinary

abnormalities (polyuria, glycosuria, ketonuria) reported in animal studies and attributed to diabetes might be due to renal tubular abnormalities. We have observed glycosuria with normoglycemia and ketonuria without ketonemia in humans given streptozotocin.

HYPOGLYCEMIC EFFECT?

There is evidence, both in animal and human studies, that streptozotocin is hepatotoxic. The role of the liver in glucose homeostasis, is, of course, of paramount importance, and may explain why severe diabetes does not occur in humans given large doses of streptozotocin.

All patients except one (No. 5) who had normal or improved glucose tolerance also had abnormal liver function tests after streptozotocin treatment. Several of these patients also had elevated one-hour growth hormone levels after treatment. Five patients with impaired glucose tolerance after treatment *also* had abnormal liver function tests and three of these also had a one-hour rise in growth hormone levels, but the degree of impaired glucose tolerance that occurred in these patients was very mild (table 3). It appears that drug-induced liver injury may modify the extent of impaired glucose tolerance that occurs with streptozotocin. Growth hormone, for example, is felt to represent an important regulatory mechanism in glucose homeostasis, and secretion may rise to offset precipitous falls in blood sugar. Streptozotocin may exert a hypoglycemic action through its effect on liver enzyme function, thus explaining the elevated growth hormone levels that occurred in some patients after treatment.

The most likely cause of hypoglycemia would be interference with hepatic glucose production or release. Streptozotocin has been shown to inhibit nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide, reduced form (NADH) in mouse liver for 48 hours (15). These pyridine nucleotides are thought to play an important role in hepatic gluconeogenesis (16); inhibition of these coenzymes may cause fasting hypoglycemia. Alternatively, streptozotocin may interfere with hepatic glucose release by inhibiting hepatic glucose 6-phosphatase. *N*-Nitrosodimethylamine (NSC-23226) is closely related to streptozotocin and has been shown to inhibit hepatic glucose 6-phosphatase in mice (17). Speculation that streptozotocin may inhibit glucose 6-phosphatase in the renal tubule and pancreatic beta cell has been pointed out previously (12).

TABLE 3.—Responses to streptozotocin therapy

Patients with—		
Normal or improved glucose tolerance after therapy	Elevated growth hormone after therapy	Abnormal liver function tests after therapy
Patient Nos. 2*, 4, 5, 6, 7, 10, 15	Patient Nos. 3, 4, 5, 6, 7, 11, 12	Patient Nos. 2, 3, 4 (LDH only), 6, 7, 8, 9, 10, 11, 12 (alk phos only), 15

*After third injection.

MISCELLANEOUS EFFECTS

There are so many variables affecting carbohydrate metabolism in man that it is difficult to isolate a single dominant effect of streptozotocin on glucose homeostasis. While streptozotocin undoubtedly destroys some of the pancreatic beta cells in man, there presumably is not the total destruction that is seen in rats. Partial loss of beta-cell function may simulate the prediabetic state where elevated blood glucose levels result in higher rates of insulin secretion. Another consideration is whether renal impairment due to streptozotocin can cause further impairment of glucose tolerance, as is suggested in this study. In addition, miscellaneous factors such as stress, hypokalemia, and weight loss may further impair glucose tolerance. While the number of patients in this series is too small to draw any conclusions, several patients in whom impaired glucose tolerance developed became hypokalemic (renal tubular defect) or lost considerable weight or both (table 2). It is altogether surprising that the degree of glucose intolerance in man produced by streptozotocin is so mild and inconstant. One possible explanation is that the drug may exert a hypoglycemic action, as mentioned previously. An alternative consideration is that the metabolism and kinetics of streptozotocin in man varies significantly from rats.

The biologic activity of streptozotocin (or any drug for that matter) would appear to be more complex in man than in a genetically homogeneous species of rats. Cancer chemotherapy in particular is a field where differential response and variable toxicity of cytotoxic agents is seen regularly. The metabolism and pharmacodynamics of streptozotocin would be likely to vary in genetically heterogeneous humans. Similarly, the mechanisms of glucose homeostasis may also vary in man. In this setting, the effect of streptozotocin on glucose homeostasis, renal function, or tumor growth in man would not be expected to be uniform.

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Clinical Investigation of a New Podophyllum Derivative, Epipodophyllotoxin, 4′-Demethyl-9-(4,6-*O*-2-thenylidene-β-D-glucopyranoside) (NSC-122819), in Patients with Malignant Lymphomas and Solid Tumors [1, 2]

Per Dombernowsky, Nis I. Nissen, and Vagn Larsen [3]

SUMMARY

Results are reported from a clinical trial of epipodophyllotoxin, 4′-demethyl-9-(4,6-*O*-2-thenylidene-β-D-glucopyranoside) (NSC-122819; VM 26), a new podophyllotoxin derivative. NSC-122819 was given intravenously twice weekly to 27 patients and intraperitoneally and topically to three patients. Since no clinical information on the compound was available, small dose increments of 0.1–0.2 mg/kg were used in the first patients. In the latter part of the study the patients were taken more rapidly to the 1–3 mg/kg level, which was considered to be the therapeutic level. Partial remission was noted in three of eight patients with Hodgkin's disease and one of thirteen patients with solid tumors. No remissions were noted in two patients with reticulum cell sarcoma or one patient with acute myeloblastic leukemia. Since only some of these patients received an adequate trial, we consider the response rate to be a clear indication of cytostatic activity of the compound. Except for one case of anaphylaxis and many cases of possibly solvent-induced chemical phlebitis at the sites of injection, the main toxic effects were hematologic. In particular, previously treated patients who had Hodgkin's disease with poor bone marrow reserve had leukopenia and thrombocytopenia, whereas patients with solid tumors tolerated larger doses.

[Cancer Chemother Rep 56:71–82, 1972]

An extract from the plant *Podophyllum emodi* (the main active constituent of which is the spindle poison podophyllotoxin) has been used since 1942 as a cytostatic agent in the topical treatment of condylomata acuminata (1). In 1963, compounds for systemic use synthesized by the Sandoz research laboratories, reached clinical trial under the designations SPI–77 and SPG–827[4] (2–5). Continued systematic research at the Sandoz laboratories has now led to a new podophyllotoxin derivative, epipodophyllotoxin, 4′-demethyl-9-(4,6-*O*-2-thenylidene-β-D-glucopyranoside) (NSC–122819), with antineoplastic characteristics which in preliminary animal experiments seem to surpass those of the earlier compounds (6,7).

A short review of the available information

[1] Received Mar 16, 1971; revised Oct 18, 1971; accepted Nov 1, 1971.

[2] NSC-122819 (VM 26) was supplied by Sandoz Ltd., Basle, Switzerland.

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[4] See List of Compounds at end of text for NSC numbers and chemical nomenclature of compounds mentioned in this paper.

on NSC–122819 will be given in this report, and our own experiences from a clinical trial in 30 patients will be presented and discussed.

CHEMISTRY

The empirical formula of NSC–122819 is $C_{32}H_{32}O_{13}S$, corresponding to a molecular weight of 656.7. The structural formula is shown in figure 1.

Since NSC–122819 is poorly soluble in water, solvents such as dimethyl sulfoxide (DMSO) plus Tween 80 have been used in some animal studies (7), and a mixture of dimethylacetamide plus cremophor EL (a mixture of ricinus-oil plus ethyleneoxide, 83:17 [BASF, Ludwigshafen, Germany]) plus ethanol has been used in other animal studies and in the clinical preparation.[5]

ANIMAL STUDIES: TOXICITY

Investigations of acute toxicity were complicated by the toxicity of the solvent.[5] The scanty information published mentions only the LD17, which with intraperitoneal (ip) administration is 51 mg/kg in mice and 52 mg/kg in rats, when DMSO plus Tween 80 is used as solvent. No animals died before 2–3 days after the injection (7). In an unpublished toxicology report from Sandoz laboratories,[5] the intravenous LD50 was 16 mg/kg for mice and 11 mg/kg for rats. These experiments differed from those mentioned above in the composition of the solvent for NSC–122819, which was more similar to that used in the clinical preparation. Most of the toxic effects were thought to be due to the solvent.

The subacute toxicity level in mice, determined as the LD17, was 5.3 mg/kg with six daily ip injections, 5.0 mg/kg with 12 daily ip injections, and 17 mg/kg with ip treatment twice weekly for 2 weeks (7).

Subacute and chronic toxic effects were also examined in rats and rhesus monkeys. Treatment 6 days a week for 4 weeks produced no deaths in rats treated with 1.8 mg/kg and a mortality rate of 12 in 20 occurred with 6.0 mg/kg.[5]

In six monkeys treated 6 days a week for 4 weeks with 0.4 mg/kg, 1.2 mg/kg, and 3.6 mg/kg, one death on Day 12 at 0.4 mg/kg was accompanied by colitis, bone marrow depression, and pneumonia. At 1.2 mg/kg both animals survived with diarrhea and moderate marrow depression. At 3.6 mg/kg both animals died (Days 13 and 27) with severe bone marrow depression, weight loss, and colitis. The results of blood chemistry tests were normal with all doses. In one monkey treated with 0.4 mg/kg of NSC-122819 and in two control animals treated with the solvent alone, no significant changes were observed; however, the injection of the solvent often elicited transitory signs of collapse and also produced local phlebitis.

It is difficult from these animal toxicity data to predict the maximal tolerated dose (MTD) in man. With application of the conversion factors calculated by Freireich et al (8) (human MTD on an mg/kg base corresponds to one twelfth the mouse LD10), the human MTD should be about 1.0 mg/kg on a twice-weekly schedule. The death of a monkey on Day 12 after a daily dose of 0.4 mg/kg suggested a human MTD of 0.1 mg/kg with daily treatment.

CELLULAR EFFECT

In cell cultures of P815 cells, KB cells, and L fibroblasts, NSC-122819 has a clear cytostatic effect with an ED50 (50% inhibition of cellular proliferation) from 0.005 to 0.012 mg/liter (7).

In fibroblast cultures Stähelin found that NSC-122819 possesses the abilities of a spindle poison as demonstrated by an immediate meta-

[5] Sandoz, Ltd. Personal communication.

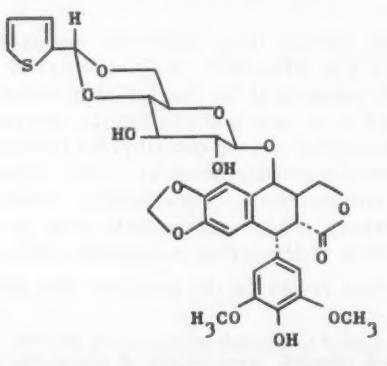

FIGURE 1.—Structural formula of NSC-122819.

phase arrest, but otherwise the drug seemed to have preprophase activity in preventing cells from entering the mitotic phase, as demonstrated by the disappearance of all mitotic figures after 120 minutes of incubation (7). The preprophase block seemed irreversible when exposed cells were washed and reincubated without the drug (7).

It was concluded that the spindle poison activity of the drug occurred at a higher concentration than the preprophase activity, but details to substantiate this conclusion were not published.

Preliminary information from the Sandoz research laboratories (7)[6] indicated that ^{3}H-thymidine incorporation in the DNA of P815 cells is inhibited 10%–15% by NSC-122819 at ED50 and 33% at ED100, whereas no effect is noted on RNA, protein synthesis, glycolysis, or respiration.

ANTITUMOR EFFECT

In Sarcoma 37, Sarcoma 180, and Walker carcinosarcoma 256, tumor weight in percent of controls was reduced to 32%–59%, 28%–58%, and 4%–32% respectively, varying according to dose, which was less than the LD17, and to treatment schedule (7).[6]

Growth of Ehrlich ascites tumor in percent of control was reduced to 0% at a dose less than the LD17, but no survivors were noted. In mice inoculated ip with 10^{6} L1210 leukemic cells, a twice-weekly schedule was claimed to be superior to daily treatment and gave a maximum increase in lifespan of 373% at a dose of 6.0 mg/kg given ip twice weekly for 4 weeks. Eight of 48 animals were 4-week survivors and were considered cured. Daily treatment gave an increase in lifespan of 246%, but since the treatment period was only 2 weeks, the results are not directly comparable to those of the twice-weekly schedule (7).

Data with larger time intervals between injections were not conclusive or suggested inferior results (7).

For mice inoculated intracerebrally with L1210 cells, only a modest increase in lifespan of 36%–56% could be obtained (7).

IMMUNOSUPPRESSION

After immunization of mice with washed sheep erythrocytes, simultaneous or delayed treatment for 5 days with NSC-122819, 1 mg/kg, elicited a profound depression of the titer of hemagglutinating antibodies.[6]

PHARMACOLOGY

After iv administration in man, 77%–89% of NSC-122819 is found bound to serum proteins. In rats given ^{3}H-labelled NSC-122819, the half-life in blood is 34 minutes for the first 2 hours and 82 minutes for the next 2-hour period. As to distribution in the body, the highest activity is shown in the liver, adrenals, intestinal wall (possibly due to incomplete removal of bile), and kidney.[6]

The excretion is rapid and takes place mainly through the bile (22% of injected iv radioactivity after one hour, 67% after 24 hours). A smaller amount of the radioactivity is found in the urine.[6]

CLINICAL STUDIES: MATERIALS AND METHODS

The 30 patients selected for the study were in an advanced and progressive stage of disease and with few exceptions they had received prior radiotherapy or chemotherapy. All of them had measurable parameters of disease and many also had biochemical abnormalities.

Details of 25 patients given systemic treatment with NSC-122819 alone are shown in table 1. Also shown are two patients receiving NSC-122819 in combination with other drugs and three patients given local or ip treatment.

The following examinations were carried out 2–3 times weekly: hemoglobin level; leukocyte, platelet, erythrocyte, reticulocyte, and differential counts; and creatinine, alkaline phosphatase, and serum glutamic oxaloacetic transaminase levels. Other examinations carried out once weekly were: serum electrophoresis, serum bilirubin, and uric acid; urinalysis for protein, blood, and sugar; and microscopy of the urine. Also, relevant radiologic examinations and bipedal lymphangiography in all the lymphoma patients were done.

NSC-122819 for clinical use was supplied in

[6] See footnote 5.

TABLE 1.—Patient status

Patient No.	Age, sex	Diagnosis	Stage or extent of disease	Duration of disease (mos)	Previous treatment and response*		Concomitant therapy and schedule	NSC–122819 schedule
1	40, M	Hodgkin's disease	IVB	61	X-ray therapy (mantle) Procarbazine Vinblastine	CR NR CR	—	Twice weekly
2	54, M	Hodgkin's disease	IVB	11	Procarbazine Prednisone X-ray therapy	CR NR CR	—	Twice weekly
3	49, F	Hodgkin's disease	IVB	5	Nitrogen mustard Cyclophosphamide	PR PR	—	Twice weekly
4	49, M	Hodgkin's disease	IVB	41	X-ray therapy (neck) Procarbazine Vinblastine Vincristine, nitrogen mustard, procarbazine, prednisone	CR CR PR PR	—	Twice weekly
5	27, M	Hodgkin's disease	IVB	43	X-ray therapy (neck) Vinblastine Nitrogen mustard	CR NR NR	—	Twice weekly
6	29, M	Hodgkin's disease	IVB	8	Vincristine, procarbazine, prednisone	PR	—	Twice weekly
7	42, F	Hodgkin's disease	IVB	33	X-ray therapy (multiple ports) Procarbazine Vinblastine Cyclophosphamide	CR CR NR PR	—	Twice weekly
8	23, F	Hodgkin's disease	IVB	48	X-ray therapy (mantle) Nitrogen mustard Vincristine, procarbazine, prednisone	CR CR PR	Prednisone, 10 mg×3	Twice weekly; later once weekly
9	56, M	Reticulosarcoma	IVB	5	Vincristine+prednisone X-ray therapy (abdomen) Vincristine+prednisone Cyclophosphamide+prednisone	PR NR NR PR	Prednisone, 10 mg×3	Twice weekly
10	68, M	Reticulosarcoma	IVB	7	X-ray therapy Procarbazine+vinblastine Cyclophosphamide+vincristine+prednisone BCNU+streptonigrin+prednisone	PR NR NR NR	—	Twice weekly
11	49, M	Acute myeloblastic leukemia	—	6	Hydroxyurea Daunomycin BCNU+cytosine arabinoside VAMP	NR NR NR NR	—	Twice weekly
12	45, F	Gastric carcinoma, inoperable	Gastric tumor; lymph node metastases	9	Exploratory laparotomy 5–FU+cytosine arabinoside	— PR	—	Twice weekly

and response to NSC–122819

NSC–122819 treatment										
Maximum single dose (mg/kg)	Total amount given (mg)	Average dose (mg/kg/wk)	Duration of treatment (days)	Response and duration	Treatment stopped due to—†	Toxicity‡				
						Hb	Wbc	Platelets	GI	Overall
2.3	2463	2.4	125	PR, 76 days	PD	2	3	1	0	3
0.5	80	0.5	22	NR	Death from progressive disease	0	0	0	0	0
1.8	965	2.1	71	PR, 60 days	PD	0	1	0	2	2
2.3	465	1.9	38	NR	PD	2	1	3	0	3
0.4	104	0.5	19	NR	PD	0	0	0	2	2
2.0	1955	3.3	53	NR	PD	2	2	0	1	2
1.0	341	0.7	60	NR	Severe anaphylaxis	1	0	0	2	4 (see text)
2.0	1415	1.0	237	PR, 90 days	PD	0	3	1	2	3
2.3	535	3.6	16	NR	Severe pancytopenia	2	4	4	1	4
0.5	65	0.5	13	NR	Death from progressive disease	0	0	0	0	0
1.9	1875	2.7	56	NR	NR	1	0	1	0	1
2.8	791	2.2	49	NR	PD	0	0	0	1	1

Patient No.	Age, sex	Diagnosis	Stage or extent of disease	Duration of disease (mos)	Previous treatment and response*		Concomitant therapy and schedule	NSC–122819 schedule
13	58, F	Gastric carcinoma	Recurrent epigastric tumor	24	Surgical treatment 5–FU	— NR	—	Twice weekly
14	64, F	Gastric carcinoma, inoperable	Nonresectable, palpable epigastric tumor	30	Surgical treatment 5–FU 5–FU+cytosine arabinoside	— NR NR	—	Twice weekly; later, once weekly
15	64, M	Gastric carcinoma	Nonresectable, palpable epigastric tumor	12	5–FU 5–FU+cytosine arabinoside	NR NR	—	Twice weekly
16	61, M	Gastric carcinoma	Recurrent epigastric tumor; cervical lymph node metastases	12	Surgical treatment 5–FU 5–FU+cytosine arabinoside	— NR NR	—	Daily
17	54, M	Gastric carcinoma	Liver and lymph node metastases	16	Exploratory laparotomy 5–FU	— NR	—	Twice weekly
18	66, M	Esophageal carcinoma	Nonresectable esophageal tumor	30	Surgical treatment Mitomycin C Methotrexate 5–FU	— NR NR NR	—	Once weekly
19	51, M	Esophageal carcinoma	Nonresectable tumor; liver and gastric metastases	3	Exploratory laparotomy	—	—	Twice weekly
20	43, F	Colon carcinoma	Liver metastases; abdominal mass; ascites	12	Surgical treatment 5–FU 5–FU+cytosine arabinoside	— NR NR	—	Twice weekly
21	67, M	Colon carcinoma	Nonresectable palpable abdominal tumor	12	Surgical treatment 5–FU	— NR	—	Twice weekly
22	63, F	Rectal carcinoma	Liver and cutaneous metastases	24	Surgical treatment	—	—	Twice weekly
23	24, M	Hepatoma	Nonresectable palpable tumor	18	Exploratory laparotomy 5–FU	— PR	—	Twice weekly
24	67, F	Ovarian carcinoma	Palpable ovarian tumor; cutaneous metastases	84	Surgical treatment SPI–77+SPG–827+ methotrexate Methotrexate Cyclophosphamide	— PR NR NR	—	Twice weekly
25	56, M	Lung carcinoma, inoperable	Lymph node metastases	6	Cyclophosphamide	NR	—	Twice weekly
26	30, M	Testicular carcinoma	Lymph node and pulmonary metastases	10	Surgical treatment X-ray therapy	— CR	Methotrexate (daily) Cyclophosphamide (daily)	Twice weekly

—Continued

NSC–122819 treatment										
Maximum single dose (mg/kg)	Total amount given (mg)	Average dose (mg/kg/wk)	Duration of treatment (days)	Response and duration	Treatment stopped due to—†	Toxicity‡				
						Hb	Wbc	Platelets	GI	Overall
1.5	488	2.3	32	NR	PD	0	0	0	0	0
2.8	1082	3.8	33	NR	PD	1	1	0	2	2
1.9	341	2.4	20	NR	PD	2	0	0	0	2
1.9	485	7.2	10	NR	PD	0	0	0	0	0
1.5	848	2.8	37	NR	PD	1	1	0	0	1
3.8	2506	2.1	211	NR	PD	0	0	0	0	0
1.1	295	1.7	23	NR	PD	0	0	0	0	0
4.0	2382	2.0	130	NR	PD	0	0	0	0	0
1.8	140	0.8	23	NR	PD	0	0	0	0	0
2.3	2941	2.2	141	NR	PD	0	0	0	1	1
4.4	6460	3.0	281	PR, 90 days	PD	0	0	0	0	0
1.4	534	2.6	26	NR	PD	0	1	0	0	1
0.2	55	0.4	22	NR	PD	0	0	0	0	0
1.0	420	2.4	19	NR	PD	0	1	0	0	1

TABLE 1.—Patient status and response to NSC–122819

Patient No.	Age, sex	Diagnosis	Stage or extent of disease	Duration of disease (mos)	Previous treatment and response*		Concomitant therapy and schedule	NSC–122819 schedule
27	54, M	Esophageal carcinoma	Nonresectable tumor; lymph node metastases	9	Exploratory laparotomy 5–FU + cytosine arabinoside	— NR	5–FU Cytosine arabinoside	Twice weekly
28	53, M	Gastric carcinoma	Liver metastases; ascites	12	Exploratory laparotomy	—	—	Ip, q5d×3
29	36, F	Gastric carcinoma	Liver metastases; ascites	36	Exploratory laparotomy	—	—	Ip, q5d×2
30	61, F	Rectal carcinoma	Cutaneous metastases	24	X-ray therapy (perineum)	NR	—	Local, q3d×5

*See List of Compounds at end of text for NSC numbers and chemical nomenclature of compounds mentioned.
CR = complete remission; PR = partial remission (> 50% regression of all measurable parameters); NR = no response.
†PD = progressive disease.
‡Toxicity ratings (relative to pretreatment blood counts): Hemoglobin (hb)—0 = normal; 1 = > 11–12.9 g/100 ml; 2 =

5-ml ampules containing 25 mg of the drug plus solvent (dimethylacetamide, 300 mg; cremophor EL, 2500 mg; and ethanol to 5 ml).

In view of the previously mentioned MTD of 0.4 mg/kg on daily treatment in monkeys, a starting dose of 0.1 mg/kg was used in our patients. After this dose, increments of 0.1–0.2 mg/kg were given twice weekly to the first five patients, bringing them to the 1.0-mg level in about 3 weeks. When this escalation elicited no toxicity, the next patients were brought to the 1.0-mg/kg level in 1 week, after which steps of 0.2–0.3 mg/kg were used (with a single exception in patient No. 9) until toxicity occurred or the patient withdrew from study due to progressive disease.

In three patients local treatment was given as described in the Results section.

RESULTS

The antitumor effects and toxic effects from treatment are summarized in table 1.

Antitumor Effect

Due to the necessary dose experiments, only some of the patients received a satisfactory trial. In the lymphoma group six of ten patients had a duration of treatment longer than 3 weeks and an intensity of treatment leading to hematologic toxicity. Among these six patients, three with Hodgkin's disease achieved partial remissions of 90, 76, and 60 days duration respectively. No effect could be observed in the other lymphoma patients.

Case Report.—Patient No. 8 was a 23-year-old woman who had had stage IVB Hodgkin's disease since 1964. Previous treatment included cyclophosphamide, several series of radiotherapy, and nitrogen mustard. In 1968, combination chemotherapy with procarbazine plus vincristine plus prednisone led to partial remission. This patient is now in relapse with inguinal lymphomas.

During treatment with NSC-122819 given twice weekly in slowly increasing doses until marrow toxicity occurred, a gradual decrease in lymphomas and improvement in general status were noted, and on Day 105 she was considered to be in partial remission. Persistent anemia prevented a complete remission rating, but when prednisone was added, a complete remission of 90 days' duration was obtained.

Antitumor effect was difficult to evaluate in one patient (No. 10) with reticulum cell sarcoma who died on Day 13 from progressive disease, while the other patient with this disease (No. 9) failed to show any response despite intensive treatment which led to pronounced pancytopenia.

One patient (No. 11) with acute myeloblastic leukemia had a leukocyte count of 55,000 with 83% blast cells and 82% marrow blast cells. Despite treatment for 8 weeks with NSC-122819 in doses up to 1.9 mg/kg, no effect on

—Continued

NSC–122819 treatment										
Maximum single dose (mg/kg)	Total amount given (mg)	Average dose (mg/kg/wk)	Duration of treatment (days)	Response and duration	Treatment stopped due to—†	Toxicity‡				
						Hb	Wbc	Platelets	GI	Overall
1.8	300	2.0	24	NR	PD	0	2	0	0	2
—	42	—	16	NR	PD	—	—	—	—	—
—	20	—	10	NR	PD	—	—	—	—	—
—	—	—	—	NR	PR	—	—	—	—	—

>9–11 g/100 ml; 3 = >7–9 g/100 ml; 4 = ≤7 g/100 ml. Wbc count—0 = normal; 1 = 3000–4900 cells/mm³; 2 = 1000–2900 cells/mm³; 3 = <1000 cells/mm³; 4 = <1000 cells/mm³ with life-threatening infection. Platelet count—0 = normal; 1 = >75,000–99,000 cells/mm³; 2 = >50,000–75,000 cells/mm³; 3 = >20,000–50,000 cells/mm³; 4 = <20,000 cells/mm³. Overall and gastrointestinal (GI)—0 = none; 1 = slight; 2 = moderate; 3 = severe; 4 = life-threatening.

peripheral blood or marrow could be observed, the wbc counts increasing to 149,000 during treatment.

Fourteen patients with metastasizing solid tumors received systemic treatment. Among these, 13 were treated for more than 3 weeks, but only in four did the treatment reach toxic levels. One patient (No. 23) obtained a partial remission of 90 days' duration without any hematologic toxicity. No antitumor effect was noted in the other patients.

Case Report.—Patient No. 23 was a 24-year-old man with a primary nonresectable metastasizing hepatoma diagnosed at laparotomy in June 1967. During treatment with 5-fluorouracil, a partial remission of 11 months' duration was obtained. In May 1968, relapse with increasing size of the liver tumor occurred. Treatment with NSC-122819 induced another partial remission on Day 150, when the twice-weekly dose had been increased to 2.8 mg/kg. During continued therapy the patient was kept in remission for 90 days, after which he relapsed despite an increase in dose to 4.4 mg/kg. Even with this large dose no toxicity was observed.

Two patients (Nos. 26 and 27) received NSC-122819 in combination with other drugs but failed to show response.

In an attempt to control ascites production in two patients with gastric carcinoma and peritoneal carcinosis, NSC-122819, 0.2 mg/kg, was administered ip at 5-day intervals. No effect was obtained. The injections caused severe and long-lasting abdominal pain, but no further toxicity.

In one patient with metastatic rectal carcinoma, topical application of NSC-122819 on an ulcerating perineal tumor was given for 2 weeks without effect.

TOXICITY

A severe anaphylactic reaction was noted in one patient (No. 7) who on Day 58 had reached a dose level of 1 mg/kg. During the infusion, chills, fever to 40 C, dyspnea, lumbar pain, and falling blood pressure developed. She recovered quickly after the cessation of NSC-122819 treatment and the start of hydrocortisone (NSC-10483) administration. Three days later a small test dose of NSC-122819 given iv immediately elicited the same symptoms and further treatment was stopped.

Dose-dependent hematologic toxicity was a common occurrence in the lymphoma group, where the patients had usually received extensive radiotherapy or chemotherapy or both, but less common in patients with solid tumors.

These results are shown in figure 2, where the curve indicates the percentage of patients with at least a grade 1 toxicity rating in wbc, platelets, or hemoglobin at each cumulative dose

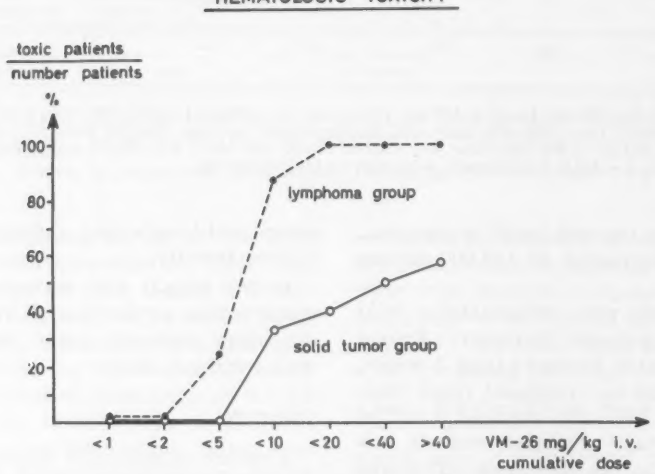

FIGURE 2.—Hematologic toxicity in adult patients after administration of NSC-122819 iv twice weekly. Patients were rated "toxic" when they showed at least a grade 1 rating in hemoglobin level or wbc or platelet counts. Since tolerance was never observed, patients with toxicity at lower doses are recorded, for graphic purposes, as showing toxicity at higher doses also, even if they stopped therapy before that level was reached. (See table 1 for definition of toxicity ratings.)

level. Toxicity was not noted until a cumulative dose between 250 and 500 mg was reached. At a dose of NSC-122819 greater than 1000 mg, all patients with lymphoma had hematologic toxicity, whereas half the patients with solid tumors had no bone marrow depression.

Further details of hematologic toxicity are shown in table 1. Patient No. 9, who was in a terminal phase of generalized reticulum cell sarcoma, had a grade 4 toxicity, the development of which is shown in figure 3. The fall in wbc and platelet counts developed rapidly, and he died with profound pancytopenia a few days after the last dose of NSC-122819. Despite the pronounced hematologic effect, no antitumor effect was noted.

Similar rapidly developing but less severe toxicity was noted in a few other patients and was the main reason for our reluctance to increase the single doses above 2–3 mg/kg, even if patients showed no response and no signs of toxicity.

Among the less severe side effects were gastrointestinal symptoms such as nausea and

vomiting in the hours after NSC-122819 infusion. This was noted in one third of the patients. Two patients also had diarrhea. The gastrointestinal symptoms never necessitated cessation of therapy.

Reversible alopecia was noted in two patients after 4 weeks of treatment.

A side effect which limits the use of NSC-122819 administered in the present solvent is chemical phlebitis at the site of injection, which occurred almost constantly. It was clearly dose-dependent, but difficult to avoid at the large doses even when the drug was infused at high dilutions. Another indication of a local tissue reaction to drug or solvent was the severe abdominal pain noted in patients given ip injections for carcinomatous ascites. Chemical phlebitis has also been noted with other podophyllum derivatives (5).

No abnormalities were noted in liver and kidney function during treatment.

DISCUSSION

The first part of this study was an exploration of dose and toxicity, since no clinical information was available to us at that time. The weekly increments in dosage were small, and

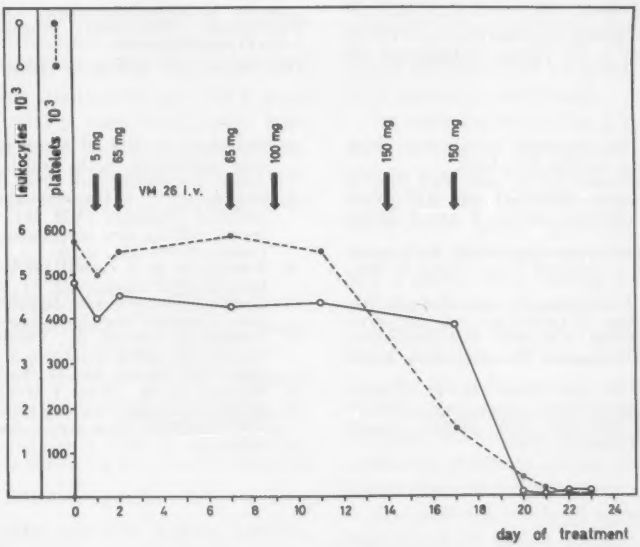

FIGURE 3.—Hematologic toxicity during NSC-122819 treatment of a patient (No. 9) with reticulum cell sarcoma in terminal state.

retrospectively, many of the first patients did not have a satisfactory therapeutic trial. In the latter part of the study the patients more rapidly reached the level of 1–2 mg/kg, which was therapeutic in some patients. But this dose level also led to rapidly developing bone marrow toxicity in some patients and we were reluctant to increase the dose further, even if this might have led to response in some of the many patients with solid tumors showing neither response nor toxicity.

With these premises in mind, three partial remissions in eight patients in the lymphoma group is a result which seems to indicate that NSC-122819 is a clinically cytostatic agent.

A recent preliminary report in abstract form supports this conclusion. Trempe et al (9) noted some response in three of four patients with Hodgkin's disease and one of two patients with reticulum cell sarcoma. Nine patients with solid tumors were all treatment failures.

In another phase I study reported in abstract form, Muggia (10) found no objective tumor regression in 17 patients (with solid tumors?) receiving weekly courses of NSC-122819. Reversible leukopenia was the only toxic manifestation.

Further investigations with larger groups of patients will be necessary to determine whether NSC-122819 is active in other categories of neoplastic disease, and whether its cytostatic activity in Hodgkin's disease is sufficient for routine clinical use. It is our impression that the almost constantly occurring, possibly solvent-induced, chemical phlebitis forms a technical problem which should be solved before more extensive use of NSC-122819.

LIST OF COMPOUNDS

BCNU: NSC-409962; urea, 1,3-bis(2-chloroethyl)-1-nitroso-
Cyclophosphamide: NSC-26271; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate; Cytoxan
Cytosine arabinoside: NSC-63878; cytosine, 1-β-D-arabinofuranosyl-, monohydrochloride; ara-C
Daunomycin: NSC-82151
5-Fluorouracil; NSC-19893; 5-FU
Hydroxyurea: NSC-32065
6-Mercaptopurine: NSC-755; purine-6-thiol, monohydrate; 6-MP
Methotrexate: NSC-740; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-
Mitomycin C: NSC-26980
Nitrogen mustard: NSC-762; diethylamine, 2,2'-dichloro-*N*-methyl-, hydrochloride
Prednisone: NSC-10023; pregna-1,4-diene-3,11,20-trione, 17,21-dihydroxy-
Procarbazine: NSC-77213; *p*-toluamide, *N*-isopropyl-α-(2-methylhydrazine)-, monohydrochloride
SPG-827: NSC-42076; podophyllotoxin, 4,6-*O*-benzylidene-β-D-glucoside
SPI-77: NSC-72274; podophyllic acid, 2-ethylhydrazide
Streptonigrin: NSC-45383
VAMP: vincristine + methotrexate + 6-mercaptopurine + prednisone
Vinblastine: NSC-49842; vincaleukoblastine, sulfate (1:1), monohydrate
Vincristine: NSC-67574; leurocristine, sulfate (1:1)

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Mycophenolic Acid (NSC-129185): Preliminary Clinical Trials [1, 2]

T. B. Brewin, [3] M. P. Cole, [4] C. T. A. Jones, [4] D. S. Platt, [5] and I. D. H. Todd [4]

SUMMARY

Thirty-seven patients with a wide range of advanced malignant tumors were treated with mycophenolic acid. In no instance was tumor regression conclusively demonstrated. Six patients experienced relief from pain. Toxic effects were negligible.

[Cancer Chemother Rep 56:83–87, 1972]

Mycophenolic acid, first detected over 70 years ago, is a substance with antibiotic activity. It has recently been extracted from the mold *Penicillium stoloniferum* and, during screening tests, was shown to have the additional property of inhibiting cell division in mammalian cell cultures. Inhibition of the growth of transplanted tumors (Sarcoma 180, Lewis lung tumor, Carcinoma 755, Walker carcinosarcoma 256, and Yoshida tumor) in rodents was subsequently demonstrated and was shown to be due to the specific inhibition of the enzyme inosinic acid dehydrogenase, whereby the formation of xanthylic acid, guanylic acid, and ultimately, the guanine nucleotides of RNA were blocked (1–3). Toxicity was uncommon, bone marrow depression with leukopenia and thrombocytopenia being rarely encountered in contrast to the experience with other anticancer drugs.

PATIENTS AND METHODS

A total of 37 patients (21 in Glasgow, Scotland, and 16 in Manchester, England) were treated with mycophenolic acid between November 1969 and July 1970 (table 1). Histologic proof of the diagnosis is available in all but four of the patients (Nos. 18, 21, 26, and 37). In most cases, all conventional forms of treatment (surgery, radiotherapy, hormones, and chemotherapy) had already been used, and the tumors had progressed or recurred after such treatment. Only patients with clinically or radiologically measurable tumor deposits were entered into the trial. At the start of the trial, the general condition of 12 of the patients (Nos. 6, 11, 14, 16, 17, 25, 28, 31, 32, 33, 34, and 36) could be described as good, all being fully mobile and well nourished.

In most patients, the drug was administered in the form of 400-mg tablets. The earlier patients started treatment at 200–400 mg daily, but later the patients were treated with 1.6 g daily, this being increased by 800-mg increments on alternate days to a maximum dose of 6.4 g daily. This higher level was attained in approximately 2 weeks.

The latter dose was not exceeded since minimal evidence of biologic activity was noted, despite the achievement of higher plasma levels (*see* next paragraph) than in those laboratory animals where tumor regression was noted. In addition, patients found it unpleasant to take more than 16 tablets daily.

Serial blood levels of mycophenolic acid were estimated in seven patients. Peak absorption occurred after 2–3 hours and although conjugation to the glucuronide and subsequent excre-

[1] Received Apr 7, 1971; revised Oct 27, 1971; accepted Nov 2, 1971.

[2] Mycophenolic acid: 4-hexenoic acid, 6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-5-phthalanyl)-4-methyl-, (*E*)-. We thank Imperial Industries Ltd. of Alderly Park, Cheshire, England, for supplying the mycophenolic acid used in these trials.

[3] The Institute of Radiotherapeutics, Glasgow, Scotland.

[4] The Christie Hospital and Holt Radium Institute, Manchester, England.

[5] Pharmaceuticals Division, Imperial Chemical Industries Ltd., Mereside, Alderley Park, Macclesfield, Cheshire, England.

TABLE 1.—Patients treated with mycophenolic acid (patients 1–21 treated in Glasgow, patients 22–37 treated in Manchester)

Patient No.	Diagnosis	Age/sex	Time from diagnosis to start of therapy (mos)	Extent of disease	Total dose (g)	Duration of treatment (days)	Days at maximum dose
1	Seminoma	27/M	15	Lungs, liver	35	39	—
2	Carcinoma, nasopharynx	45/M	23	Lungs, bone, skin	125	63	14
3	Carcinoma, breast	69/F	157	Skin, pleura	14	22	—
4	Fibrosarcoma	19/M	26	Lungs, skin	424	101	51
5	Hodgkin's disease	37/M	60	Lungs, bone	365	142	34
6	Carcinoma, ovary	60/F	15	Ascites	252	130	35
7	Carcinoma, breast	52/F	60	Bone, skin, liver	31	29	—
8	Reticulum cell sarcoma	61/M	61	Nodes, bone	322	104	31
9	Carcinoma, breast	52/F	21	Lungs, skin	285	90	45
10	Hodgkin's disease	30/F	75	Lungs, bone	14	13	—
11	Secondary carcinoma (primary unknown)	37/M	36	Bone	315	81	27
12	Carcinoma, breast	53/F	67	Bone, skin	95	47	16
13	Teratoma, testis	28/M	38	Lungs	144	84	49
14	Carcinoma, breast	51/F	24	Chest wall	294	70	14
15	Carcinoma, breast	48/F	40	Lungs	23	7	—
16	Carcinoma, breast	65/F	57	Skin, nodes	206	43	22
17	Glioblastoma	47/M	4	Brain	194	39	28
18	Glioma, spinal cord	47/M	32	Cervical spinal cord	348	101	14
19	Carcinoma, thyroid	61/M	102	Ulcerated nodes	362	63	56
20	Hemangioblastoma (cerebellum)	43/M	240	Brain	340	77	28
21	Carcinoma, bronchus	47/M	8	Nodes, skin, liver	90	15	15
22	Carcinoma, breast	40/F	36	Skin, lungs	139	28	16
23	Carcinoma, uterine cervix	29/F	12	Cervix, lymph nodes	267	49	33
24	Carcinoma, sigmoid colon	31/F	4	Sigmoid colon, peritoneum	87	56	1
25	Malignant melanoma	51/F	2	Skin, lungs	177	38	18
26	Carcinoma, rectum	69/M	12	Rectum peritoneum	529	90	76
27	Carcinoma, bladder	49/M	15	Bladder, skin, lymph nodes	350	65	44
28	Fibrosarcoma, thigh	75/M	1	Muscle, skin	235	146	33
29	Carcinoma, breast	35/F	36	Breast, bone	51	14	—
30	Carcinoma, uterine cervix	55/F	12	Cervix, lymph nodes, liver	265	51	28
31	Neurofibrosarcoma, neck	62/M	24	Skin	163	84	10
32	Malignant melanoma	60/F	12	Skin, lymph nodes	14	9	—
33	Squamous cell carcinoma, bronchus	60/M	36	Lung	265	84	—
34	Carcinoma, breast	55/F	144	Breast, skin	128	41	2

TABLE 1.—Patients treated with mycophenolic acid (patients 1–21 treated in Glasgow, patients 22–37 treated in Manchester)—Continued

Patient No.	Diagnosis	Age/sex	Time from diagnosis to start of therapy (mos)	Extent of disease	Total dose (g)	Duration of treatment (days)	Days at maximum dose
35	Liposarcoma, abdominal wall	44/M	6	Skin	165	33	19
36	Malignant melanoma, uterine cervix	60/F	24	Cervix, vagina, rectum	29	10	—
37	Carcinoma, bronchus	55/M	5	Lung	107	41	2

tion proceeded rapidly, blood levels were maintained for up to 6 hours after a single oral dose of 400 mg (fig 1). Proportionately higher plasma levels were produced by an increase in oral dose (*see* "Analysis of Free and Conjugated Mycophenolic Acid in Human Plasma," next section). Urine levels were not estimated.

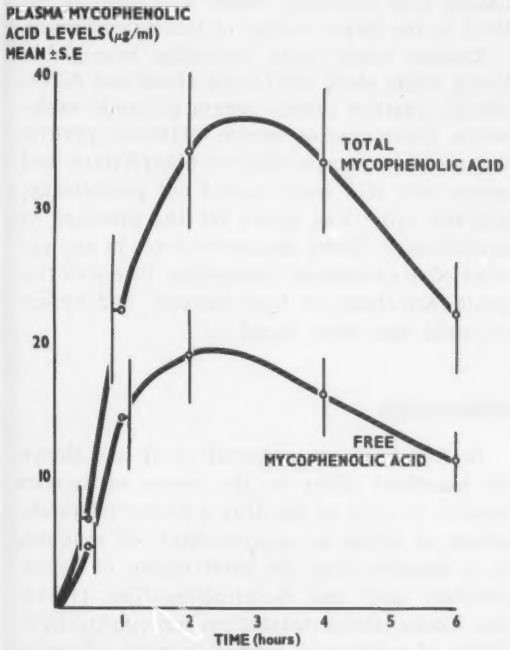

FIGURE 1.—Mean plasma concentrations of mycophenolic acid after ingestion of one 400-mg tablet (7 patients).

The period of administration of the drug varied from 7 to 142 days (mean, 57 days) while the total amount taken varied between 14 and 529 g (mean, 194 g). Twenty-eight patients were able to tolerate the higher doses of 6.0 or 6.4 g daily for periods ranging from 1 to 76 days (mean, 31 days).

A number of the patients received other drugs in addition to mycophenolic acid during the trial. Courses of 5-fluorouracil (to a total dose of 6 g over 12 days) were administered twice to patient No. 24, and 6-mercaptopurine (150 mg daily) was given for one week to patient No. 27. Seven patients (Nos. 1, 5, 22, 24, 29, 35, and 37) received oral corticosteroids, and in one patient (No. 36), prednisolone was administered rectally, concurrently with mycophenolic acid.[6]

ANALYSIS OF FREE AND CONJUGATED MYCOPHENOLIC ACID IN HUMAN PLASMA

Free mycophenolic acid is extracted from human plasma as follows: To plasma (1.0 ml) in a stoppered test tube, add 1 ml distilled water, 1 ml 0.1 M acetate buffer (pH 5.0), 1 ml 0.05 N HCl, and ether (12 ml). Extract the drug by shaking the tube for 5 minutes. The solvent layers are separated by slow-speed centrifugation, and a 10-ml aliquot of the ether phase re-extracted with 3 ml 0.1 N NaOH.

The concentration of mycophenolic acid in the alkaline extract is determined by spectro-

[6] 5-Fluorouracil: NSC-19893.
6-Mercaptopurine: NSC-755; purine-6-thiol, monohydrate.
Prednisolone: NSC-9900; pregna-1,4-diene-3,20-dione, 11β,17,21-trihydroxy-.

photofluorimetry (activation at 345 nm, emission at 430 nm, uncorrected), by comparison with reference standards prepared by subjecting control plasma (with known amounts of drug added) to an identical extraction procedure.

Total (free and conjugated) mycophenolic acid is determined as follows: The method is identical to that indicated for free acid except that the acetate buffer (1 ml) contains 5000 units β-glucuronidase (5 mg Helocase[R] β-glucuronidase). Before addition of 0.05 N HCl and extraction into ether, the tubes are incubated for 24 hours at 37 C to hydrolyze the glucuronide.

Control plasma, to which amounts to mycophenolic acid-glucuronide are added, are treated in the same way to provide reference standards.

The recovery of free mycophenolic acid from plasma is 85%, with a coefficient of variation not exceeding 5% on replicate analyses in the plasma concentration range of 0.5–20 μg/ml. The extent of hydrolysis of the glucuronide between batch assays varied from 50% to 70% but the inclusion of the reference standards with each batch allowed this variability to be adequately controlled.

The "total" level of mycophenolic acid in figure 1 is the sum of the free concentration and that derived from the glucuronide by hydrolysis, ie, concentration of mycophenolic acid-glucuronide (μg/ml plasma) $= \frac{(T-F)}{0.623}$ where T = "total" concentration and F = free concentration.

RESULTS

No measurable tumor regression was noted in any patient. One man (No. 37) with bronchogenic carcinoma showed a marginal diminution in radiographic shadowing, though this coincided with a temporary response of his superimposed chest infection to antibiotic therapy. A reduction in pain was reported by six patients (Nos. 6, 8, 11, 26, 30, and 31), accompanied in three patients by reduced demand for analgesics. One patient (No. 5) reported relief of generalized pruritus and two (Nos. 9 and 19) had less bleeding from ulcerated areas.

TOXIC EFFECTS

In two patients (Nos. 1 and 24) stomatitis with buccal ulceration developed, accompanied in one patient by interdigital desquamation of the hands. There was no accompanying leukopenia in either patient. In both, the severity of the reaction seemed to be dose dependent, there being no further stomatitis after reduction of mycophenolic acid dosage. In one patient (No. 10) a severe paranoid confusional state developed while she was receiving the drug, and another (No. 34) complained of "mental irritability." Transiently increased pain at the tumor site was mentioned by four patients (Nos. 5, 9, 30, and 34). In one patient (No. 36) who had malignant melanoma involving the cervix and rectovaginal septum, rectal hemorrhage increased during mycophenolic acid treatment requiring blood transfusion, but ceased when the drug was discontinued. Six patients (Nos. 23, 25, 29, 32, 33, and 36) complained of difficulty in swallowing the tablets with subsequent nausea and vomiting, which was possibly related to the large number of tablets consumed.

Routine blood tests including hemoglobin levels, white blood cell counts (total and differential), platelet counts, serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, serum alkaline phosphatase, and serum uric acid were carried out periodically, and the urine was tested for the presence of urobilinogen. There was no evidence in any patient of drug-induced leukopenia, thrombocytopenia, hemolysis, or liver damage, and hyperuricemia was never noted.

DISCUSSION

In this trial, mycophenolic acid has shown no beneficial effect in the range of tumors treated. In view of the drug's distinctive mechanism of action in experimental cell systems, it is possible that the combination of mycophenolic acid and 6-mercaptopurine (which also blocks purine metabolism through the inhibition of a different enzyme, namely hypoxanthine-guanine phosphoribosyl transferase) may be effective (1).

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Clinical Experience with Dromostanolone Propionate (NSC-12198) in Breast Carcinoma [1, 2]

D. G. Seay, J. D. Bradshaw, and N. T. Nicol [3,4,5]

SUMMARY

Twenty-two patients with locally advanced or metastatic breast cancer, were treated with dromostanolone propionate at a dose of 100 mg intramuscularly once weekly. All patients were either postmenopausal or had had menopause induced. Five of 12 patients (41%) who had had no previous hormonal therapy responded. Ten patients receiving secondary therapy failed to respond, giving an overall response rate of five in 22 (22%). Side effects were few and not severe.

[Cancer Chemother Rep 56:89–92, 1972]

During the past 26 years androgenic agents have been used to treat metastatic breast carcinoma, and although they have been particularly recommended for perimenopausal patients, reasonable therapeutic results can also be obtained postmenopausally. Since Fels (1) first reported the use of testosterone propionate in breast cancer, other similar compounds have been examined in the hope of finding one which is more effective and less masculinizing. Huggins et al (2) found that a variant of testosterone (NSC-9700), 17β-hydroxy-2α-methyl-5α-androstan-3-one (NSC-26198), was a potent inhibitor of transplantable fibroadenomata in rats. This was followed by the observations of Blackburn et al (3), Goldenberg et al (4), and Thomas et al (5), that in double-blind trials with equal doses given intramuscularly (im) (100 mg, 3 times weekly), dromostanolone propionate was at least as effective as testosterone propionate (NSC-9166), if not more so, in treating postmenopausal women with metastatic breast carcinoma, and was up to 4 times less androgenic. Segaloff (6), summarizing the studies of the Cooperative Breast Cancer Group, reported that 25 of 96 menopausal and postmenopausal women had responded objectively to dromostanolone propionate, and for a period, this compound given 3 times weekly was adopted as a standard reference compound by the trial groups.

Using tritium-labelled dromostanolone propionate, Gual et al (7) demonstrated that after a single weekly injection of 100 mg im, there was a continuous accumulation of free radioactive metabolites in the plasma with each dose. This effect was partly due to slow absorption from the injection site. They postulated that effective treatment could be carried out by giving 100 mg im at intervals of 7–10 days. We, therefore, decided to use dromostanolone propionate at doses of 100 mg im weekly, and this paper describes our results with this regimen.

METHODS AND SELECTION OF PATIENTS

Patients with objective evidence of disease progression were selected for study. In no case was dromostanolone propionate used prophy-

[1] Received Apr 30, 1971; revised Aug 3, 1971.

[2] Dromostanolone propionate: 5α-androstan-3-one, 17β-hydroxy-2α-methyl-, propionate; Masteril.

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[5] We wish to thank Dr. E. M. Ward for his interpretation of the slides, Mr. J. Grenfell for the photographs, and especially Dr. G. A. Christie of Syntex Pharmaceuticals Limited for his advice and for supplies of dromostanolone propionate.

lactically. No premenopausal (natural or induced) patients were included, and it was felt prudent to exclude patients with rapidly progressing metastases. All types of metastases, however, including a mixed picture, and all histologic variants of breast carcinoma were considered suitable for inclusion.

Baseline biochemical studies, radiographs, photographs, and objective measurements were taken initially, for reference at followup examinations. Histologic confirmation of metastases was obtained where necessary, for example in early chest-wall recurrence, and in some cases repeated later to confirm response. All patients were given dromostanolone propionate (100 mg im) once weekly, and the cases were assessed by two or more observers at intervals of 2–4 weeks depending on the patient's physical state. Treatment was continued for 2–3 months at least, unless a patient's deteriorating condition contraindicated this. The latter occurred particularly in those who had had previous hormone therapy, and who were followed more closely.

A response was accepted if there was significant measurable regression of at least half of the metastatic lesions while the others remained static, but was not accepted if any lesions enlarged or a new metastasis appeared. Patients whose metastases remained static ("doubtful") were considered nonresponders.

RESULTS

A total of 22 patients were treated for an average of 4.5 months with a range of 1–12 months. Five (22%) responded to treatment, which was continued for an average of 7.5 months.

PRIMARY TREATMENT

Dromostanolone propionate was the primary hormone alteration in 12 of 22 patients whose average age was 65. One 65-year-old patient was only 2 years postmenopausal and did not respond. The remaining 11 patients were all 5 years or more beyond their natural menopause, and all five responders (average age, 63 years) fell within this group, giving a 45% response rate or an overall rate of 41% for all patients receiving this as initial treatment for their metastases.

Three of the five responders had local soft tissue recurrence and the other two had mixed metastases (one soft tissue and visceral, the other bone and visceral). Of the seven nonresponders, three had soft tissue disease, one had visceral cancer (pulmonary, which remained static initially), and the remaining three had mixed disease, including two with bony metastases. Two patients with liver enlargement, one confirmed as metastatic at laparotomy 6 months earlier, and the other highly suggestive on clinical grounds, tolerated the hormone as assessed clinically for 5.5 and 12 months.

SECONDARY TREATMENT

Ten of the 22 patients received dromostanolone propionate as secondary treatment after previous hormone therapy. Eight of these patients (average age, 43 years) had oophorectomy or X-ray ovarian ablation and were still within 5 years of their menopause. The remaining two patients were 60 and 63 years old. None of these ten responded to dromostanolone propionate and treatment was discontinued after an average of 2.5 months, when there were clear objective signs of advancing cancer.

SIDE EFFECTS

In no case and at no stage was treatment abandoned because of intolerable side effects. Although hirsutism and a husky voice appeared in six of 22 patients, usually 3–9 months after the start of therapy and mainly in the "primary treatment" group, their effects were invariably minimal (table 1). Plethora, seborrhea, altered libido, or hypercalcemia did not occur.

In only one patient in the secondarily treated group did anything of note develop—a pronounced degree of facial hirsutism appearing after 9 weeks. Four years earlier pronounced hirsutism and a husky voice developed in this patient, who was 4 years post-oophorectomy, after treatment with nandrolone decanoate, 50 mg given 3 times weekly for 3 months before

TABLE 1.—Side effects in primary treatment group

Patient No.	Months of treatment with dromostanolone propionate	Response	Side-effects	Time of toxicity onset (mos)	Comment
1	2	No	None	—	—
2	12	Yes	Husky voice	8	Visibly thickened cord
			Ankle edema	9	Slight
			Hirsutism	9	Slight
3	9	No	None	—	Treatment continued as a doubtful response
4	3	No	Husky voice	2.5	—
5	1	No	None	—	Mixed picture of metastases; continued to deteriorate
6	5½	No	Restlessness	—	Occurred 2–3 days after injection
			Hirsutism	5	Face and legs
7	6	Yes	Acne	5	Slight
8	6	No	None	—	Treatment continued initially while pulmonary metastases static
9	5	Yes	Hirsutism	3	Slight
			Husky voice	3	Slight
10	5	Yes	Husky voice	4	Slight
			Hirsutism	4	Slight
11	5	No	None	—	—
12	10	Yes	Hirsutism	5	Slight
			Husky voice	5	Slight

oophorectomy.[6] With dromostanolone propionate, the degree of vocal change was clearly less pronounced. Subjective improvement in the sense of enhanced well being occurred in six of 22 patients, but was not a striking phenomenon. In nine patients from either group treated for more than 2 months and followed after cessation, no withdrawal response was noted.

DISCUSSION

The principal finding of this patient study is that the response rate to dromostanolone propionate is at least as good when the drug is given at this lower dose for primary hormone therapy as in the previously reported series where higher doses were used. Because of the small size of the primary group, their average age of 65 years being well beyond the menopause and all of them receiving this as their first treatment, no particular significance can be attached to the actual regression rate obtained, particularly as the latter two factors tend to favor a good hormonal response. Nevertheless, these findings do support the encouraging results previously reported. When giving dromostanolone propionate as secondary therapy, we were unable to confirm the 19% regression rate reported by Witt et al (8). This total lack of response was probably due to the advanced stage of the disease in almost all of these patients, to their proximity to menopause, and to the fact that many had had more

[6] Nandrolone decanoate: estr-4-en-3-one, 17β-hydroxy-, decanoate; Decadurabolin.

than one trial of hormone treatment previously, without an initial response. It may, however, have been due to the lower dose used.

Previously, authors have stated that dromostanolone propionate was less androgenic, dose for dose, than testosterone propionate. Our experience, admittedly with lower doses, confirms this and indicates that the side effects that do occur are mild and unlikely to hinder treatment. Thickened vocal cords and hirsutism were the most common effects, the latter being problematic in only one patient.

Despite prolonged therapy, two patients with liver enlargement tolerated dromostanolone propionate clinically, and in a third patient with more advanced disease, icterus never developed although her condition was deteriorating. These results confirm those of Blackburn et al (3).

The androgenicity of dromostanolone propionate, although difficult to measure, is likely to be related to the amount and frequency of administration. It is, therefore, interesting to speculate on a dosage scheme. As stated earlier, Gual et al (7) showed that with 100 mg im once weekly there were greater concentrations of radioactivity in the plasma after each injection. This suggested slow elimination, which was corroborated by urinary studies, in addition to slow absorption from the injection site. Therefore, after three or more 100-mg doses in the first 2 weeks it might be possible to maintain a patient with 100 mg every 7–10 days or even 150–200 mg given once every 2 weeks.

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Evaluation of 1,3-Bis(2-chloroethyl)-1-nitrosourea (BCNU; NSC-409962) in the Management of Patients With Advanced Breast Cancer [1, 2]

David L. Ahmann, Richard G. Hahn, and Harry F. Bisel [3, 4]

SUMMARY

Sixteen patients with advanced breast cancer were treated with 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) at a dose of 75–100 mg/m² on each of 3 successive days. No therapeutic responses were observed. The death of two patients 4 weeks after the start of treatment was attributed to the drug; in the other 14 patients toxic effects were present but acceptable. This study failed to demonstrate that BCNU is of value in the management of patients with end-stage breast cancer. The effect of earlier application was not assessed.

[Cancer Chemother Rep 56:93–94, 1972]

1, 3 - Bis (2 - chloroethyl) - 1 - nitrosourea (BCNU) has shown appreciable antitumor effect against a number of neoplasms in man (1); however, it has not had an adequate clinical trial in patients with advanced breast cancer. The purpose of this study was to assess the therapeutic effectiveness of BCNU, with or without the addition of prednisone,[5] in patients who had advanced breast cancer.

MATERIALS AND METHODS

Sixteen patients with advanced breast cancer were chosen for this study. No other more effective therapy was available to these patients, 12 of whom had viscerally dominant disease. All patients had had previous hormonal therapy, which either had been effective initially and failed subsequently or had never been effective. All patients had previously had an adequate clinical trial with one or more of the other cytotoxic chemotherapeutic agents and 13 of the 16 patients had previously had alkylating therapy with cyclophosphamide.[6]

Seven patients were initially given 100 mg/m² of BCNU on each of 3 successive days, but subsequent toxicity resulted in dosage alteration to 75 mg/m² on each of 3 successive days in the remaining nine patients. All patients were randomized as to the addition of prednisone, which was given orally in doses of 30 mg/day for 2 weeks, 20 mg/day for 1 week, and a maintenance dose of 10 mg/day.

Before therapy, all patients had adequate hematologic status with white blood cell (wbc) counts of more than 4000 cells/mm³ and platelet counts of more than 100,000 cells/mm³. None of the patients had received significant radiation therapy to regions containing bone marrow that might preclude cytotoxic chemotherapy. Wbc counts were obtained twice weekly and platelet counts weekly for 8 weeks after therapy or until the counts were well within normal range.

[1] Received May 25, 1971; revised Aug 3, 1971.
[2] NSC-409962 was supplied by the Cancer Therapy Evaluation Branch, Chemotherapy, National Cancer Institute (NCI).
[3] Mayo Clinic and Mayo Foundation, Rochester, Minn.
[4] The authors wish to thank Dr. Stephen K. Carter, Chief, Cancer Therapy Evaluation Branch, Chemotherapy, NCI, for his assistance.
[5] Prednisone: NSC-10023; pregna-1,4-diene-3,11,20-trione, 17,21-dihydroxy-.

[6] Cyclophosphamide: NSC-26271; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate; Cytoxan.

Since no responses were noted in either therapy group, the study was abandoned after the 16 patients had been treated. Patients were given only one course of BCNU, because the disease had progressed at the time of subsequent re-evaluation.

RESULTS

All 16 patients under study had progressive metastatic disease 8 weeks after completion of therapy with BCNU. One patient showed evidence of a clinical response after 4 weeks of treatment, but subsequent evaluation at 8 weeks revealed that the tumor was larger than when the patient entered the study.

The toxicity patterns of this agent were similar to those previously reported (1). Two drug deaths were reported. Both patients had been receiving 75 mg/kg/day for 3 successive days and both deaths occurred 4 weeks after treatment was started. The deaths were septic deaths related to leukopenia.

Nine of the 16 patients had leukopenia (wbc <3000 cells/mm^3) and nine had thrombopenia (platelet count $<100{,}000$ cells/mm^3). The nadir of the leukopenia was 33 days and that of the thrombopenia was 27 days. The duration of hematopoietic depression varied from 3 to 14 days. Ten of the 16 patients had nausea and vomiting, but the gastrointestinal distress was not severe enough to warrant the parenteral use of fluids.

At the time of this report, eight of the 16 patients have died, with a median survival of 2.5 months and a mean survival of 2.8 months after therapy. Eight patients are alive 10 months, 9 months, 6 months, 5 months, and 2 months (four patients) after therapy.

COMMENT

BCNU is not a valuable agent for the treatment of patients with end-stage breast cancer. It is unlikely that further courses of BCNU would have been beneficial, since all patients had progressive metastatic disease 8 weeks after treatment.

The results of a phase II study such as this are undoubtedly influenced by the far-advanced stage of the disease in the patients selected, and by the previous exposure to and failure of other cytotoxic chemotherapeutic agents.

Within 3 months after treatment, 50% of the patients died (although the drug contributed to death in two patients); however, while the other 50% are alive at the time of this report (mean, 4.7 months), all 16 patients failed to benefit, either initially or subsequently, from previous cytotoxic chemotherapy.

The search for therapeutic agents effective in end-stage breast cancer should continue. Unfortunately, the patients in this category tolerate vigorous cytotoxic chemotherapy poorly. Future phase II studies, if their purpose is to screen new agents that might be effective against a particular neoplasm, should include patients with earlier disease.

BCNU may or may not be clinically effective in patients with an earlier stage of disseminated breast cancer. This study merely demonstrated that this agent is ineffective in treating patients with end-stage advanced breast cancer.

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Phase II Study of Camptothecin (NSC-100880) in the Treatment of Advanced Gastrointestinal Cancer[1, 2, 3]

Charles G. Moertel, Allan J. Schutt, R. J. Reitemeier, and R. G. Hahn [4]

SUMMARY

Camptothecin was used in the treatment of 61 patients who had advanced gastrointestinal adenocarcinoma. Thirty-four patients were treated with a single injection administered every 3 weeks at initial doses ranging between 90 and 180 mg/m². Twenty-seven patients were given 5-day courses repeated every 4 weeks at initial total doses of 55–110 mg/m². Toxic effects included nausea, vomiting, diarrhea, stomatitis, dermatitis, alopecia, hemorrhagic cystitis, leukopenia, thrombocytopenia, and anemia. Only two of the 61 patients (3%) had partial objective responses after 2 months.

[Cancer Chemother Rep 56:95–101, 1972]

Camptothecin is an alkaloid with a unique heterocyclic ring structure (1). It is notable for its exotic derivation from the wood of *Camptotheca acuminata*, a tree native to the rocky slopes of north China. Initial evidence of antineoplastic activity was obtained in the screening of a large number of natural products by Drug Research and Development (formerly Cancer Chemotherapy National Service Center), National Cancer Institute (NCI). It was later found to have significant activity in mouse leukemia L1210 and rat Walker 256 carcinosarcoma. The possibility of usefulness against gastrointestinal carcinoma was originally raised because of the specific toxic effect of camptothecin in the intestinal mucosa of monkeys and beagles. This interest was intensified in 1970 by the phase I study of a group at the NCI Baltimore Cancer Research Center (1). In this investigation eight of nine patients with large bowel cancer were reported to have objective response.

The present study was undertaken in cooperation with the Chemotherapy Program of the NCI, to assess in greater detail the possible use of camptothecin in the treatment of advanced gastrointestinal cancer.

MATERIALS AND METHODS

Patient Selection

The 61 patients selected for study all had adenocarcinoma known to be gastrointestinal in origin. The sites of the primary lesions are listed in table 1. All patients had unresectable metastatic cancer proved by histologic examination, and all had measurable areas of known cancer to serve as objective indicators of response to therapy. The age and sex distribution, the location of the indicator lesions, and the unassociated disease states are shown in table 2. All were ambulatory outpatients and all were maintaining a reasonable state of hydration and nutrition before treatment. Three patients had significant fluid retention before treatment, three had mild elevation of serum creatinine levels (1.2–1.5 mg), and one patient had mod-

[1] Received June 25, 1971, accepted Aug 18, 1971.
[2] Supported by contract NIH-70-2066 from Chemotherapy, National Cancer Institute (NCI).
[3] NSC-100880 was supplied by Drug Research and Development, Chemotherapy, NCI.
[4] Division of Gastroenterology and Internal Medicine (Drs. Moertel, Schutt, and Reitemeier) and Division of Clinical Oncology and Internal Medicine (Dr. Hahn), Mayo Clinic and Mayo Foundation, Rochester, Minn.

erate azotemia secondary to ureteral obstruction.

Thirty patients had had no previous treatment for their advanced cancer. Seven had had radiation therapy to the abdomen or pelvis, and 28 had had earlier chemotherapy, primarily with 5-fluorouracil (NSC-19893) (table 3). No patient had had either chemotherapy or radiation therapy for at least one month before camptothecin therapy, and all had returned to a normal peripheral hematologic status. Fifty-two patients received additional drugs for symptomatic and supportive purposes either immediately before or during the course of treatment (table 4).

Methods

All patients were instructed to drink 8 ounces of water every waking hour and to pass urine every hour for 24 hours before treatment, during treatment, and for 48 hours after treatment. Parenteral fluids were administered if there was clinical evidence of dehydration during therapy.

The planned dose of camptothecin was dissolved in physiologic saline at a concentration of 2 mg/ml and administered by rapid intravenous infusion over 5–10 minutes. Two schedules of administration were used: (*a*) a single injection repeated at 3-week intervals; and (*b*) a 5-day course repeated every 4 weeks. Our initial dose for the single-dose method was 180 mg/m² based on the suggestions by Gottlieb and his co-workers (1). Because of toxic effects we considered excessive, later patients were treated at doses ranging between 90 and 120 mg/m². Dosages for the 5-day course were based on work reported by Muggia (2) and ranged between 11 and 22 mg/m²/day (total course, 55–110 mg/m²). Leukocyte and platelet counts were obtained at least twice weekly after the start of therapy, and appropriate prophylactic and supportive measures were started for

TABLE 1.—Site of origin of primary adenocarcinoma

Primary site	No. of patients
Colon	34
Rectum	15
Stomach	6
Pancreas	2
Appendix	1
Bile duct	1
Esophagus	1
Small bowel	1

TABLE 2.—Patient characteristics

Sex	
Male: 26	
Female: 35	
Age	
Mean: 57 yrs	
Median: 58 yrs	
Range: 28–78 yrs	
Primary indicator lesions	
Hepatic	21 patients
Pulmonary	17 patients
Cutaneous or subcutaneous	13 patients
Abdominal	9 patients
Nodal	1 patient
Unassociated diseases	
Coronary insufficiency	3 patients
Hypertension	3 patients
Diabetes mellitus	2 patients
Gout	1 patient

TABLE 3.—Previous therapy

Therapy	Patients
None	30
Radiation	7
Cytotoxic drugs*	28
5-Fluorouracil	22
CCNU	9
Emetine	3
BCNU	2
Streptozotocin	2
Cyclophosphamide	1

*CCNU: NSC-79037; urea, 1-(2-chloroethyl)-3-cyclohexyl-1-nitroso-.
Emetine: NSC-33669; emetine, hydrochloride.
BCNU: NSC-409962; urea, 1,3-bis(2-chloroethyl)-1-nitroso-.
Streptozotocin: NSC-85998.
Cyclophosphamide: NSC-26271; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate.

TABLE 4.—Concomitant drug therapy*

Drug	No. of patients	Drug	No. of patients
Aspirin	22	Caffeine	12
Narcotics	22	Barbiturates	8
Prochlorperazine	22	Diazepam	7
Propoxyphene	21	Codeine	4
Phenacetin	14	Methyprylon	4

*27 other drugs given to 3 or fewer patients each.

patients experiencing severe marrow depression. For all subsequent courses with each method it was our objective to raise or lower the dosage for each patient to obtain a level of definite but clinically tolerable toxicity. Patients receiving single-dose therapy were re-evaluated every 3 weeks and patients receiving the 5-day course were re-evaluated every 4 weeks. This re-evaluation consisted of a medical history, physical examination, measurement of indicator lesions, and repetition of appropriate laboratory studies. Treatment regimens were administered at least twice to each patient if their general condition permitted, and for those who showed objective improvement or who remained objectively stable, treatment was continued until advancing disease was evident.

RESULTS

Toxicity

The toxic reactions to the first course of camptothecin therapy are documented in tables 5 and 6 according to the method of administration and dose used.

Nausea and vomiting were usually coincident with drug administration, but would occasionally persist for 1–2 weeks after treatment.

Diarrhea was only a problem at higher doses, but then could be quite severe to the point of fecal incontinence and persistent for as long as 4 weeks. In one drug-related fatality, inflammation and multiple ulcers were found throughout the ileum and large bowel.

Cystitis usually began about 7–10 days after treatment and was characterized clinically by dysuria and frequency. With more severe toxicity, gross hematuria developed. Pathologically, this was characterized by multiple necrotic ulcerations which could involve the entire urinary tract from kidney pelvis to bladder. The occurrence of hemorrhagic cystitis did not preclude further treatment with camptothecin, and its

TABLE 5.—Toxic reactions: single-dose method

Dose (mg/m^2)	No. of patients treated	*Nonhematologic* No. of patients with— Nausea	Vomiting	Diarrhea	Stomatitis	Cystitis	Alopecia	Dermatitis
90	10	3	2	—	1	1	8	—
100	6	3	3	—	1	2	2	—
110	2	2	1	1	—	1	2	—
120	7	5	3	4	—	2	6	—
180	9	8	5	2	2	3	6	—

Dose (mg/m^2)	No. of patients treated	*Hematologic* No. of patients with— Leukopenia, nadir <4000 >2000	Leukopenia, nadir <2000	Thrombocytopenia, nadir <150,000 >100,000	Thrombocytopenia, nadir <100,000 >50,000	Thrombocytopenia, nadir <50,000	Anemia (fall in hgb level >2 g/100 ml)
90	10	2*	—	2*	—	—	—
100	6†	1	1	1	—	1	—
110	2	1	—	1	—	—	1
120	7	2*	2	1‡	—	—	1
180	9	2	4	1	2	1	3

*In 1 patient counts were incomplete.
†Drug-related death.
‡In 3 patients counts were incomplete.

TABLE 6.—Toxic reactions: 5-day course

Nonhematologic

Daily dose ($mg/m^2 \times 5$)	No. of patients treated	No. of patients with—						
		Nausea	Vomiting	Diarrhea	Stomatitis	Cystitis	Alopecia	Dermatitis
11	2	1	1	—	—	1	—	—
15	9	6	4	1	2	4	5	—
17	5	4	3	4	—	2	4	—
20	10	8	4	4	1	6	8	1
22	1	1	1	1	—	—	1	1

Hematologic

Daily dose ($mg/m^2 \times 5$)	No. of patients treated	No. of patients with—					
		Leukopenia, nadir		Thrombocytopenia, nadir			Anemia (fall in hgb level >2 g/100 ml)
		<4000 >2000	<2000	<150,000 >100,000	<100,000 >50,000	<50,000	
11	2	—	—	—	—	—	—
15	9*	1†	2	1†	—	1	1
17	5	—	—	1	1	—	2
20	10*	2	3	3	2	1	6
22	1	1	—	—	—	1	1

*Drug-related death.
†In 1 patient counts were not adequately followed.

severity could be titrated down by lowering the dose in subsequent courses.

Stomatitis, similar to that encountered with actinomycin D (NSC-3053), was only a problem in patients experiencing profound toxic effects.

Two patients had giant urticaria developing within 2 days after the completion of 5-day courses. There was almost always some degree of alopecia which persisted for as long as treatment was continued.

We found no evidence of renal toxicity. No severe hepatotoxicity was observed but five patients had transient rises in serum glutamic oxaloacetic transaminase (SGOT) levels. One patient with rectal carcinoma had an acute episode of left upper quadrant pain associated with a significant rise in urine amylase excretion, and one additional patient showed a similar amylase rise in the absence of any symptoms of pancreatitis. Screening studies did not reveal any evidence of induced coagulopathy, adrenal toxicity, or alterations of calcium metabolism.

The dose-limiting toxicity of camptothecin was bone marrow depression affecting all cell lines. This occurred early, with unpredictable severity, and frequently very precipitously (table 7). Recovery was usually prompt but in several patients, particularly those initially treated with large doses, we found evidence of cumulative marrow toxicity with later courses. Three patients died after profound hematologic toxic reactions, two after treatment with 5-day courses and one after a single injection. Each of these patients had normal serum creatinine levels immediately before treatment, but one was found at necropsy to have pelvic carcinoma producing bilateral ureteral obstruction with hydronephrosis. One had pretreatment evidence of significant fluid retention.

Comparison of the toxicity spectrums of the two methods of treatment (table 8) showed that with dosage spreads producing a comparable degree of vomiting, stomatitis, alopecia, and leukopenia, the 5-day course was associated with more frequent diarrhea, cystitis, throm-

TABLE 7.—Patterns of hematologic toxicity

Treatment		Leukopenia	Thrombocytopenia
Single-dose method			
Day of Nadir:	Mean	12.3	11.0
	Range	7–20	8–18
Duration (days):	Mean	9.5	5.4
	Range	1–19	1–10
5-Day course			
Day of Nadir:	Mean	16	14.2
	Range	8–22	12–16
Duration (days):	Mean	5.2	2.8
	Range	1–11	1–8

TABLE 8.—Relationship of method of administration to toxicity spectrum

Toxic reaction	Method of administration: Single dose (% of 34 patients)	Method of administration: 5-Day course (% of 27 patients)
Vomiting	41	41
Diarrhea	21	37
Stomatitis	12	11
Cystitis	24	48 ($P<0.05$)
Dermatitis	—	7
Alopecia	71	67
Leukopenia: <4000 cells/mm³	47	35
<2000 "	22	19
Thrombocytopenia: <100,000 cells/mm³	13	23
<50,000 "	7	12
Anemia: fall in hgb level >2 g/100 ml	15	39 ($P<0.05$)

bocytopenia, and anemia. The more prolonged schedule produced more severe toxicity at a given total dose level, but the difference was not as great as might have been predicted by preclinical animal studies (3).

We feel a reasonable initial dose is 110–120 mg/m² for the single-injection method or 17 mg/m²/day (total dose, 85 mg/m²) for the 5-day course. Even at these moderate doses, however, severe drug reactions may occur.

Therapeutic Response

Our objective evaluation of response in the 61 patients treated is documented in table 9. We have defined an objective response for mass lesions as a 50% reduction in the product of perpendicular diameters of the most clearly measurable area of known cancer. There may be no increase in any other areas of known disease, and no new areas of cancer may develop. If malignant hepatomegaly is quantitated for objective response, there must be a decrease of at least 30% in the sum of measurements below the xyphoid process and the costal margins at the left and right midclavicular lines. Measurements are made with quiet respiration. Laboratory assessments of liver function (serum bilirubin, alkaline phosphatase, and SGOT) must remain stable or show improvement.

After 2 months (8 or 9 weeks) only two of our 61 patients showed evidence of partial objective improvement, and none showed improve-

TABLE 9.—Objective results

Single-dose method (34 patients)

Objective results*	Time after start of therapy			
	3 wks	6 wks	9 wks	12 wks
Improved	4	2	2	—
Stable	17	11	8	6
Worse	13	21	24	28

5-Day course (27 patients)

Objective results	Time after start of therapy		
	4 wks	8 wks	12 wks
Improved	1	—	—
Stable	12	7	6
Worse	14	20	21

*3 patients showed 25%–50% response at 3 wks only.

ment at 3 months. Both patients who demonstrated an objective response at 2 months had large bowel cancer, and both were treated with the single-injection method. If we liberalized our criteria to include those patients who showed a 25% reduction, only three additional patients would be categorized as improved and these only at 3 weeks.

Since measurement of indicator lesions in gastrointestinal carcinoma may be less than precise, particularly for abdominal masses and hepatomegaly, we also assessed several other less specific parameters of therapeutic response (table 10). Of those patients with significant pretreatment symptoms, 14% claimed symptomatic improvement but none became completely asymptomatic. Only four patients claimed improvement in performance status, and two gained weight. Of 40 patients who had pretreatment laboratory evidence of impaired liver function, three showed improvement of all abnormalities (table 11).

TABLE 10.—General parameters of response at 2 months

	Percent response of 61 patients		
	Improved	Stable	Worse
Symptoms	14	14	72
Performance status	6.5	20	74
Weight gain (±2.5%)	4	33	63

CONCLUSIONS

Camptothecin is a drug of protean and unpredictable toxicity that has no clinical value in the management of gastrointestinal cancer.

TABLE 11.—Liver function studies at 2 months (patients with initial abnormalities)

	Improved	Stable	Worse
Total bilirubin (±1 mg)	1	1	4
Alkaline phosphatase (±10 IU)	6	4	27
SGOT (±5 IU)	4	6	16

All abnormalities improved	3 patients
All unchanged	3 "
Mixed response	16 "
All worse	18 "

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Treatment of Malignant Melanoma With Camptothecin (NSC-100880) [1, 2, 3]

Jeffrey A. Gottlieb and James K. Luce [4,5]

SUMMARY

Fifteen patients with advanced malignant melanoma were treated with camptothecin at doses of 90–360 mg/m² repeated every 2 weeks. None of the patients had a 50% or greater decrease in tumor diameter. Less pronounced transient tumor regression was noted in three patients, but no clinical benefit was associated with these responses. The remaining patients had no change or progression in their disease. Toxic effects included myelosuppression (11 patients), nausea and vomiting (9), alopecia (8), diarrhea (3), and hemorrhagic cystitis (1). We conclude that camptothecin, at least as administered in this study, has little to offer the patient with advanced disseminated melanoma.

[Cancer Chemother Rep 56:103–105, 1972]

Camptothecin, an alkaloidal extract of the heartwood of the oriental tree *Camptotheca acuminata*, has shown considerable antitumor activity in several rodent tumor systems (1,2). In the preliminary clinical evaluation of this drug, one patient with malignant melanoma achieved a 5-month partial remission (3). To further evaluate the efficacy of camptothecin in this disease, we have treated 15 patients who had advanced malignant melanoma.

METHODS AND MATERIALS

Nine men and six women (median age, 48 years; range, 28–72 years) with widely disseminated disease were treated from December 1970 to April 1971. Of the 15 patients, 14 had involvement of the lung, liver, or central nervous system. Ten were refractory to previous chemotherapy with 5-(3,3-dimethyl-1-triazeno)-imidazole-4-carboxamide (NSC-45388), hydroxyurea (NSC-32065), 1,3-bis(2-chloroethyl)-1-nitrosourea (NSC-409962, BCNU), or vincristine [leurocristine, sulfate (1:1); NSC-67574] (table 1).

Camptothecin was administered as a single rapid intravenous (iv) injection starting at a dose of 120 mg/m² repeated at 2-week intervals. The dose in subsequent courses was increased by increments of 60 mg/m² per dose (to a maximum of 360 mg/m²) in eight patients who tolerated their initial doses with minimal toxicity. One patient required reduction of his dose to 90 mg/m². A total of 46 doses were administered, with 12 of the 15 patients receiving three or more doses (range, 1–5 doses). To prevent the known bladder toxicity of this drug (3,4), patients were well hydrated for 3 days after therapy. Those treated as outpatients were especially instructed by our nursing department to insure adequate oral fluid intake, and patients who failed to maintain adequate hydration orally were encouraged to return to the hospital for iv fluids. Complete hemograms and urinalyses were performed 3 times weekly together with weekly tumor measurements and renal and liver function studies. Appropriate radiologic

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[2] Supported by grants CA-10376 and CA-10379 from the National Cancer Institute (NCI).
[3] Camptothecin was supplied by Drug Research and Development, Chemotherapy, NCI.
[4] Department of Developmental Therapeutics, University of Texas M. D. Anderson Hospital and Tumor Institute, Houston, Texas.
[5] The authors gratefully acknowledge the assistance of Katherine Herrington, R. N., and Teresa Busiek, R. N.

investigations were repeated at monthly intervals or less.

RESULTS

None of the 15 patients demonstrated a greater than 50% decrease in tumor diameter. Less pronounced tumor regression was noted in three patients (two with pulmonary and one with hepatic metastases), but in none of these did the response last longer than 1 month. No clinical benefit was associated with these responses. Of the remaining 12 patients, eight had

TABLE 1.—Patient data

Patient No.	Age, sex	Prior chemotherapy	Sites of metastatic involvement	Dose (mg/m^2)	Number of courses	Response	Toxicity	Survival from start of chemotherapy
1	35, F	None	Brain	120	1	Not evaluable	None	7½ mos
2	48, M	BCNU, NSC-45388, vincristine	Brain, lungs, bone, subcutaneous lesions	120	1	Not evaluable	Leukopenia, vomiting	½ mo
3	34, M	BCNU, NSC-45388, vincristine	Lungs, nodes	120 180	1 2	Progressive disease	Alopecia	6½ mos
4	46, F	BCNU, NSC-45388, vincristine, hydroxyurea	Lungs, scalp	120	3	<50% decrease in lung metastases	Leukopenia, thrombocytopenia, anemia, vomiting, alopecia	7+ mos
5	68, F	BCNU, NSC-45388, vincristine	Lungs, soft tissue, nodes	120	3	Progressive disease	Leukopenia, vomiting, diarrhea	6 mos
6	60, F	BCNU, NSC-45388, vincristine, hydroxyurea	Lungs, subcutaneous lesions	120 180	3 1	<50% decrease in lung metastases	Leukopenia, thrombocytopenia, nausea	5½ mos
7	28, M	None	Liver, bones, nodes	120 180	1 2	Progressive disease	Leukopenia, alopecia, vomiting	5 mos
8	48, F	BCNU, NSC-45388, vincristine	Nodes, skin	120	3	Progressive disease	Leukopenia, alopecia, anemia	6+ mos
9	48, M	Vincristine	Abdomen, lungs	120 90	1 1	No change	Leukopenia, thrombocytopenia, anemia, vomiting	2 mos
10	42, F	Hydroxyurea	Lungs, nodes, soft tissue	120	3	Progressive disease	Leukopenia	2 mos
11	55, M	None	Lungs, liver	120 180 240	1 1 2	<50% decrease in liver metastases	Alopecia, diarrhea	5+ mos
12	44, M	None	Lungs, nodes	120 180	2 2	Progressive disease	Vomiting, diarrhea	4+ mos
13	72, M	BCNU, NSC-45388, vincristine, hydroxyurea	Lungs, soft tissue	120 180	1 2	Progressive disease	Leukopenia, thrombocytopenia, anemia, alopecia	5+ mos
14	56, M	None	Lungs	120 180 240 360	1 1 1 1	No change	Alopecia, leukopenia, nausea	4+ mos
15	63, M	BCNU, NSC-45388, vincristine	Lungs	120 180	2 3	Progressive disease	Alopecia, vomiting, leukopenia. cystitis	5+ mos

steadily progressive disease, two had no change in their status, and two could not be evaluated. The median survival time from the start of chemotherapy for all 15 patients was 5+ months (range, 0.5–7+ months) with seven patients still living.

Toxic reactions included myelosuppression (11 patients), nausea and vomiting (9), alopecia (8), diarrhea (3), and hemorrhagic cystitis (1). All three of the formed elements of the blood were depressed. A fall in the white blood cell count (wbc) to less than 4000 cells/mm^3, which was noted in 24 of the 46 courses (11 patients), was the most consistent hematologic toxic effect. The median wbc nadir was 2600 (range, 700–7500) and occurred between the 8th and 14th day after the start of therapy. Thrombocytopenia and anemia were each noted in only four patients but required packed red cell and platelet transfusions in each instance. There were two episodes of nonfatal pneumonia occurring during the period of myelosuppression, both of which were seen in patients whose pulmonary metastases showed slight regression. Occult blood in the stools developed in three patients during treatment, but no frank hemorrhages occurred. Vomiting and diarrhea were severely debilitating in four patients, forcing cessation of therapy in two of the four. Hemorrhagic cystitis developed in only one patient during the study, and this patient had become severely dehydrated secondary to vomiting while receiving his 5th course of drug at home.

DISCUSSION

Since the minimal responses we observed were achieved only with considerable toxicity, we conclude that camptothecin, at least as administered in this study, has little to offer the patient with advanced disseminated melanoma. It is possible that patients with less advanced disease might be more responsive to this agent, but because of the severe toxicity observed, we have replaced camptothecin with other agents in our search for effective therapy for malignant melanoma. However, the reproducibility of leukocyte depression noted by us and others (3–6) and the encouraging studies in murine L1210 leukemia (1) suggest that camptothecin may be useful in the treatment of human leukemia. A study utilizing camptothecin in this disease is currently in progress. Congener synthesis of this compound in an attempt to find a less toxic, more effective agent may be another fruitful avenue of future research.

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5-Fluorouracil (5-FU; NSC-19893), 5-(3,3-Dimethyl-1-triazeno)imidazole-4-carboxamide (NSC-45388), Vincristine (NSC-67574), and 1,3-Bis(2-chloroethyl)-1-nitrosourea (BCNU; NSC-409962) Given Concomitantly in the Treatment of Solid Tumors in Man [1, 2, 3]

Errol B. van Eden, Geoffrey Falkson, J. J. van Dyk, A. M. van der Merwe, and H. C. Falkson [4]

SUMMARY

Forty-six patients were treated with a combination of 5-fluorouracil, 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide, vincristine, and 1,3-bis(2-chloroethyl)-1-nitrosourea, given in courses once a month. Forty-three of these cases were evaluable. The side effects encountered were tolerable, consisting of decreases in hemoglobin level in 24 patients, leukopenia in 20, and thrombocytopenia in 11. Gastrointestinal side effects included nausea, vomiting, and occasional diarrhea. Alopecia was observed in all patients receiving more than one course of treatment. Good objective responses occurred in six of 12 patients with breast cancer, six of eight patients with colon carcinoma, one of seven patients with rectal carcinoma, two of two patients with cholangiocellular carcinoma, one of six patients with adenocarcinoma of unknown origin, two of three patients with ovarian carcinoma, and one patient with myelomatosis. Both the quality and the quantity of responses noted are most encouraging and further trials with the same drug combination are indicated in gastrointestinal carcinoma, ovarian carcinoma, and breast cancer.

[Cancer Chemother Rep 56:107–147, 1972]

Both 5-fluorouracil (5-FU) and vincristine have been used for many years in the treatment of patients with solid tumors. 1,3-Bis(2-chloroethyl)-1-nitrosourea (BCNU) has been of value in treating a variety of solid tumors in man (1–4). Moertel and Reitemeier showed that BCNU also had activity against gastrointestinal carcinoma (5). 5-(3,3-Dimethyl-1-triazeno)-imidazole-4-carboxamide (NSC-45388) has been used in the treatment of patients with malignant melanoma (6). It has also been shown to have antitumor activity against a variety of solid tumors in man (7).

Combination chemotherapy has proved to be of value in treating acute leukemia, testicular tumors, Hodgkin's disease, and breast cancer (8–11). The present study was undertaken to investigate the possible advantages of combining four drugs (5-FU, NSC-45388, vincristine, and BCNU) in the treatment of patients with solid tumors. All four agents differ in their mechanism of action and preliminary trials indicated that the combination would be tolerable.

[1] Received July 19, 1971, revised Sept 8, 1971.
[2] Supported in part by a grant from the National Cancer Association of South Africa.
[3] Vincristine: leurocristine, sulfate (1:1). NSC-45388 and BCNU were supplied by Drug Research and Development, Chemotherapy, National Cancer Institute.
[4] Department of Cancer Chemotherapy, H. F. Verwoerd Hospital and the University of Pretoria, South Africa.

TABLE 1.—

Patient No. (age, sex)	Performance status*	Diagnosis and findings at start of experimental therapy (ET) (duration of disease at start of ET)	Previous treatment†	Combination therapy				
				Drug	From	To	No. of doses/ total dose	
1 (64, F)	3	Ovarian carcinoma: mesenteric infiltration and ascites; palpable abdominal tumor (35 mos)	Cyclophosphamide	5-FU	5/19/69	5/23/69	5/2500	mg
				NSC-45388	5/19/69	5/22/69	4/800	mg
				Vincristine	5/19/69	—	1/2.0	mg
				BCNU	5/19/69	—	1/100	g
2 (58, M)	2	Adenocarcinoma, unknown primary: pulmonary and bone metastases ¶ (8 mos)	Radiotherapy+5-FU	5-FU	5/19/69	5/23/69	5/2500	mg
					6/16/69	6/20/69	5/2500	"
					7/21/69	7/25/69	5/3000	"
							15/8000	"
				NSC-45388	5/19/69	5/22/69	4/800	mg
					6/16/69	6/19/69	4/800	"
					7/21/69	7/24/69	4/800	"
							12/2400	"
				Vincristine	5/19/69	—	1/2.0	mg
					6/16/69	—	1/2.0	"
					7/23/69	—	1/2.0	"
							3/6.0	"
				BCNU	5/19/69	—	1/160	g
					6/16/69	—	1/160	"
					7/23/69	—	1/200	"
							3/520	"
3 (28, M)	4	Rectal carcinoma: infiltration of pelvic organs; colectomy (24 mos)	1. Surgical treatment 2. 5-FU 3. Mitomycin C+ vincristine	5-FU	6/ 2/69	6/ 5/69	4/1800	mg
				NSC-45388	6/ 2/69	6/ 5/69	4/720	mg
				Vincristine	6/ 2/69	—	1/1.5	mg
				BCNU	6/ 2/69	—	1/150	g
4 (48, F)	2	Breast carcinoma: bone metastases of pelvis, lumbar and dorsal vertebrae (23 mos)	1. Surgical treatment 2. Radiotherapy 3. Cyclophosphamide+ dromostanolone propionate 4. 5-FU+fluoxymesterone 5. Methotrexate 6. 6-Methylprednisone	5-FU	6/ 9/69	6/13/69	5/2500	mg
				NSC-45388	6/ 9/69	6/12/69	4/800	mg
				Vincristine	6/ 9/69	—	1/1.8	mg
				BCNU	6/ 9/69	—	1/150	g

Patient data

Fall in hgb level (g/100 ml)	Side effects: Leukopenia: Lowest wbc ($\times 10^3$/mm^3)	Side effects: Leukopenia: Duration of (days)	Side effects: Thrombocytopenia: Lowest platelet count ($\times 10^5$/mm^3)	Side effects: Thrombocytopenia: Duration of (days)	Time of maximum hemapoietic depression	Nausea/ vomiting‡	Diarrhea‡	Response§: Comment
2.2	2.8	—	159	—	Day 26	+/−	−	Imp: Patient died 39 days after start of ET.
2.5	5.0	—	140	—	Day 32	+/−	++	PR (2 mos): Patient died on Day 292. Autopsy showed a primary bronchus carcinoma of the left lung.
—	—	—	—	—	—	+/−	−	NE: Patient died on Day 5.
0.7	7.1	—	60	14	Day 4	+/−	−	PD: Patient died on Day 22.

TABLE 1.—

Patient No. (age, sex)	Performance status*	Diagnosis and findings at start of experimental therapy (ET) (duration of disease at start of ET)	Previous treatment†	Combination therapy			
				Drug	From	To	No. of doses/total dose
5 (64, F)	2	Breast carcinoma: bilateral breast infiltration and skin metastases; ulceration of skin of both breasts ** (32 mos)	1. Radiotherapy 2. Diethylstilbestrol+5-FU 3. Cyclophosphamide+diethylstilbestrol 4. Methotrexate+fluoxymesterone 5. 5-FU+6-methyl prednisone	5-FU	6/ 6/69	6/12/69	5/3750 mg
					7/25/69	7/30/69	4/2250 "
					9/ 5/69	9/11/69	4/3000 "
							13/9000 "
				NSC-45388	6/ 6/69	6/11/69	4/1200 mg
					7/25/69	7/29/69	3/700 "
					9/ 5/69	9/11/69	4/1200 "
							11/3100 "
				Vincristine	6/ 6/69	—	1/2.4 mg
					7/25/69	—	1/2.4 "
					9/ 5/69	—	1/2.4 "
							3/7.2 "
				BCNU	6/ 6/69	—	1/140 g
					7/25/69	—	1/140 "
					9/ 5/69	—	1/140 "
							3/420 "
6 (51, F)	3	Myelomatosis: multiple osteolytic lesions of entire skeleton and pathological fractures of ribs; abnormal protein band in gamma area of electrophoretic pattern (30 mos)	1. Phenylalanine mustard+6-methylprednisone 2. BCNU 3. Radiotherapy 4. Cyclophosphamide+6-methylprednisone	5-FU	6/ 9/69	6/13/69	5/2500 mg
					6/24/69	6/28/69	3/1500 "
							8/4000 "
				NSC-45388	6/ 9/69	6/12/69	4/800 mg
					6/24/69	6/28/69	3/600 "
							7/1400 "
				Vincristine	6/ 9/69	—	1/1.5 mg
					6/24/69	—	1/1.5 "
							2/3.0 "
				BCNU	6/ 9/69	—	1/120 g
					6/24/69	—	1/100 "
							2/220 "
7 (54, F)	3	Carcinoma, unknown origin: metastases of skull, right proptosis and papilledema (3 mos)	None	5-FU	6/ 5/69	6/11/69	5/2500 mg
				NSC-45388	6/ 5/69	6/10/69	4/720 mg
				Vincristine	6/ 5/69	—	1/2.0 mg
				BCNU	6/ 5/69	—	1/150 g

Patient data—Continued

Fall in hgb level (g/100 ml)	Side effects: Leukopenia: Lowest wbc ($\times 10^3$/mm^3)	Side effects: Leukopenia: Duration of (days)	Side effects: Thrombocytopenia: Lowest platelet count ($\times 10^3$/mm^3)	Side effects: Thrombocytopenia: Duration of (days)	Time of maximum hemapoietic depression	Nausea/vomiting‡	Diarrhea‡	Response§: Comment
2.5	4.9	—	133	—	Day 52	+/−	−	PR (3 mos): Patient died on Day 268. Autopsy showed metastases in lungs and embolis of liver.
6.1	1.5	14	30	Thrombocytopenia at start of ET	Day 18	+/+	++	PR (2 mos): Abnormal band in gamma area decreased from 2.02 g/100 ml on Day 1 to 0.57 g/100 ml on Day 81. Bedridden patient returned to normal activities.
—	—	—	—	—	—	−/−	−	NE: Patient died on Day 12.

TABLE 1.—

Patient No. (age, sex)	Performance status*	Diagnosis and findings at start of experimental therapy (ET) (duration of disease at start of ET)	Previous treatment†	Combination therapy				
				Drug	From	To	No. of doses/ total dose	
8 (71, F)	3	Cholangiocellular carcinoma: hepatomegaly (11 mos)	5-FU+cytosine arabinoside	5-FU	6/ 9/69	6/16/69	5/2500	mg
					8/14/69	8/20/69	5/2500	"
							10/5000	"
				NSC-45388	6/ 9/69	6/15/69	4/800	mg
					8/14/69	6/19/69	4/800	"
							8/1600	"
				Vincristine	6/ 9/69	—	1/1.5	mg
					8/14/69	—	1/1.5	"
			N				2/3.0	"
				BCNU	6/ 9/69	—	1/150	g
					8/14/69	—	1/150	"
							2/300	"
9 (38, F)	2	Breast carcinoma: multiple pulmonary and bone metastases †† (58 mos)	1. Surgical treatment 2. Radiotherapy 3. Oophorectomy 4. Cyclophosphamide 5. Methotrexate 6. Cyclophosphamide 7. 5-FU 8. CB-3210 9. 5-FU+methotrexate +6-methylprednisone+fluoxymesterone 10. Vincristine+ L-phenylalanine mustard+ fluoxymesterone	5-FU	6/16/69	6/20/69	5/2500	mg
					7/21/69	7/25/69	5/2500	"
					8/25/69	8/29/69	5/2500	"
							15/7500	"
				NSC-45388	6/16/69	6/19/69	4/800	mg
					7/21/69	7/24/69	4/800	"
					8/25/69	8/28/69	4/800	"
							12/2400	"
				Vincristine	6/16/69	—	1/1.7	mg
					7/21/69	—	1/1.7	"
					8/25/69	—	1/1.7	"
							3/5.1	"
				BCNU	6/16/69	—	1/100	g
					7/21/69	—	1/100	"
					8/25/69	—	1/100	"
							3/300	"
10 (43, F)	2	Breast carcinoma: infiltration of contralateral breast with skin metastases and bone metastases of lumbar vertebrae (11 mos)	1. Surgical treatment 2. Oophorectomy 3. Radiotherapy 4. Cyclophosphamide 5. Cyclophosphamide+ fluoxymesterone	5-FU	6/24/69	6/30/69	5/2350	mg
				NSC-45388	6/24/69	6/30/69	4/690	mg
				Vincristine	6/24/69	—	1/1.6	mg
				BCNU	6/24/69	—	1/135	g
11 (58, F)	2	Breast carcinoma: skin and pulmonary metastases (69 mos)	1. Surgical treatment 2. Radiotherapy 3. Diethylstilbestrol+ cyclophosphamide 4. 5-FU+diethylstilbestrol 5. Fluoxymesterone+ 5-FU	5-FU	6/13/69	6/19/69	5/2750	mg
				NSC-45388	6/13/69	6/18/69	4/800	mg
				Vincristine	6/13/69	—	1/2.0	mg
				BCNU	6/13/69	—	1/150	g

Patient data—Continued

	Side effects							
	Leukopenia		Thrombocytopenia					
Fall in hgb level (g/100 ml)	Lowest wbc ($\times 10^3$/mm^3)	Duration of (days)	Lowest platelet count ($\times 10^3$/mm^3)	Duration of (days)	Time of maximum hemapoietic depression	Nausea/ vomiting‡	Diarrhea‡	Response§: Comment
3.4	5.0	—	125	—	Day 15	−/−	++	PR (3 mos): Hepatomegaly decreased and jaundice cleared for 3 mos. Treatment stopped on Day 131 because of progressive disease
1.4	4.5	—	123	—	Day 24	+/−	−	PR (3 mos): Treatment stopped on Day 92 because of progression of pulmonary metastases.
4.6	3.2	4	30	28+	Day 24	+/−	−	Imp (1 mo): Patient died on Day 52. Lesions improved by more than 50% for 1 mo then progressed before recovery of thrombocytopenia.
1.1	2.8	8	110	—	Day 28	−/−	−	NC: Treatment stopped on Day 69 because of progressive disease.

TABLE 1.—

Patient No. (age, sex)	Performance status*	Diagnosis and findings at start of experimental therapy (ET) (duration of disease at start of ET)	Previous treatment†	Combination therapy			
				Drug	From	To	No. of doses/ total dose
12 (32, F)	2	Breast carcinoma: bone metastases ‡‡ (50 mos)	1. Surgical treatment 2. Radiotherapy 3. Oophorectomy 4. 5–FU 5. 5–FU+fluoxymesterone 6. Cyclophosphamide+fluoxymesterone 7. Methotrexate 8. Fluoxymesterone 9. 6–Methylprednisone	5–FU	6/13/69 7/25/69 9/12/69 10/31/69	6/19/69 7/31/69 9/18/69 11/ 6/69	5/2500 mg 5/2500 " 5/2500 " 5/2500 " 20/10,000 "
				NSC–45388	6/13/69 7/25/69 9/12/69 10/31/69	6/18/69 7/30/69 9/17/69 11/ 5/69	4/800 mg 4/800 " 4/800 " 4/800 " 16/3200 "
				Vincristine	6/13/69 7/25/69 9/12/69 10/31/69	— — — —	1/1.5 mg 1/1.5 " 1/1.5 " 1/1.5 " 4/6.0 "
				BCNU	6/13/69 7/25/69 9/12/69 10/31/69	— — — —	1/100 g 1/100 " 1/100 " 1/100 " 4/400 "
13 (57, F)	2	Breast carcinoma: pulmonary metastases (30 mos)	1. Surgical treatment 2. Cyclophosphamide 3. Surgical treatment 4. 5–FU+fluoxymesterone 5. 5–FU+6–methylprednisone 6. Methotrexate+6–methylprednisone 7. Mitomycin C+6–methylprednisone	5–FU	6/13/69 8/ 4/69 9/29/69 12/ 5/69	6/19/69 8/ 8/69 10/ 3/69 12/11/69	5/2500 mg 5/2500 " 5/2500 " 5/2500 " 20/10,000 "
				NSC–45388	6/13/69 8/ 4/69 9/29/69 12/ 5/69	6/18/69 8/ 7/69 10/ 2/69 12/10/69	4/800 mg 4/800 " 4/800 " 4/800 " 16/3200 "
				Vincristine	6/13/69 8/ 4/69 9/29/69 12/ 5/69	— — — —	1/2.0 mg 1/2.0 " 1/2.0 " 1/2.0 " 4/8.0 "
				BCNU	6/13/69 8/ 4/69 9/29/69 12/ 5/69	— — — —	1/150 g 1/100 " 1/100 " 1/150 " 4/500 "
14 (62, M)	2	Rectal carcinoma: liver metastases (2 mos)	Surgical treatment	5–FU	6/25/69	6/27/69	3/1650 mg
				NSC–45388	6/25/69	6/27/69	3/600 mg
				Vincristine	6/25/69	—	1/1.8 mg
				BCNU	6/25/69	—	1/150 g

Patient data—Continued

Fall in hgb level (g/100 ml)	Side effects: Leukopenia: Lowest wbc ($\times 10^3$/mm^3)	Leukopenia: Duration of (days)	Thrombocytopenia: Lowest platelet count ($\times 10^3$/mm^3)	Thrombocytopenia: Duration of (days)	Time of maximum hemapoietic depression	Nausea/ vomiting‡	Diarrhea‡	Response§: Comment
5.1	2.5	14	109	—	Day 22	+/−	−	PR (1 mo): Osteolytic lesions became more sclerotic and performance status improved for 1 mo. Treatment stopped on Day 146 because of progression of bone metastases.
3.5	3.7	5	35	14	Day 34	+/−	−	PR (3 mos): Treatment stopped on Day 218 because of progression of pulmonary metastases.
3.3	2.7	5	83	4	Day 6	+/−	+	Imp: Treatment discontinued on Day 3 because of hemapoietic depression. Patient died on Day 29. Autopsy showed massive liver metastases.

Patient No. (age, sex)	Performance status*	Diagnosis and findings at start of experimental therapy (ET) (duration of disease at start of ET)	Previous treatment†	Combination therapy			
				Drug	From	To	No. of doses/ total dose
15 (38, F)	2	Breast carcinoma: metastases of skull, pelvis, right scapula (18 mos)	1. Methotrexate 2. Cyclophosphamide	5-FU	6/27/69	7/ 3/69	5/2500 mg
				NSC-45388	6/27/69	7/ 2/69	4/800 mg
				Vincristine	6/27/69	—	1/1.8 mg
				BCNU	6/27/69	—	1/100 g
16 (73, F)	2	Breast carcinoma: skin metastases (82 mos)	1. Surgical treatment 2. Radiotherapy 3. Cyclophosphamide+ diethylstilbestrol 4. 5-FU+fluoxymesterone 5. Methotrexate 6. Fluoxymesterone 7. Cyclophosphamide+ fluoxymesterone 8. 5-FU+dromostanolone propionate 9. BCNU	5-FU	6/27/69	7/ 3/69	5/2500 mg
				NSC-45388	6/27/69	7/ 2/69	4/800 mg
				Vincristine	6/27/69	—	1/1.8 mg
				BCNU	6/27/69	—	1/150 g
17 (58, F)	4	Corpus carcinoma: palpable abdominal masses (36 mos)	1. Radiotherapy 2. Surgical treatment 3. Cyclophosphamide+ medroxyprogesterone acetate 4. 5-FU+medroxyprogesterone acetate 5. Mitomycin C+ medroxyprogesterone acetate 6. 5-FU+medroxyprogesterone acetate	5-FU	12/10/69	12/12/69	3/1500 mg
				NSC-45388	12/10/69	12/12/69	3/600 mg
				Vincristine	12/10/69	—	1/1.3 mg
				BCNU	12/10/69	—	1/100 g

Patient data—Continued

Side effects								
	Leukopenia		Thrombocytopenia					
Fall in hgb level (g/100 ml)	Lowest wbc ($\times 10^3$/mm^3)	Duration of (days)	Lowest platelet count ($\times 10^3$/mm^3)	Duration of (days)	Time of maximum hemapoietic depression	Nausea/ vomiting‡	Diarrhea	Response§: Comment
0.1	6.0	—	80	7	Day 7	−/−	−	PD: Patient died on Day 25 because of progressive disease.
4.9	5.0	—	88	9	Day 29	+/−	−	PD: Treatment stopped on Day 46 because of progression of skin metastases.
2.5	3.3	12	123	—	Day 20	++/++	−	PD: Abdominal obstruction developed on Day 4. Patient died on Day 48. Autopsy showed massive abdominal tumor with infiltration into small and large bowel.

TABLE 1.—

Patient No. (age, sex)	Performance status*	Diagnosis and findings at start of experimental therapy (ET) (duration of disease at start of ET)	Previous treatment†	Combination therapy				
				Drug	From	To	No. of doses/ total dose	
18 (65, F)	4	Ovarian carcinoma (adenocarcinoma): lung metastases with pleural effusion and peritoneal metastases§§	1. Surgical treatment 2. Cyclophosphamide 3. 5-FU	5-FU	2/10/70 4/ 3/70 5/25/70 7/14/70	2/13/70 4/10/70 5/28/70 7/15/70	4/2000 5/2500 5/2500 2/1000	mg " " "
							16/8000	"
				NSC-45388	2/10/70 4/3 /70 5/22/70 7/14/70	2/13/70 4/ 9/70 5/27/70 7/15/70	4/800 4/800 4/800 2/400	mg " " "
							14/2800	"
				Vincristine	2/10/70 4/ 3/70 5/22/70 7/14/70	— — — —	1/1.6 1/1.5 1/1.5 1/1.5	mg " " "
							4/6.1	"
				BCNU	2/10/70 2/10/70 5/22/70 7/14/70	— — — —	1/100 1/100 1/100 1/100	g " " "
							4/400	"
19 (66, F)	2	Rectal carcinoma: vaginal infiltration (40 mos)	1. Surgical treatment 2. Radiotherapy	5-FU	7/ 7/70	7/10/70	4/3000	mg
				NSC-45388	7/ 7/70	7/ 8/70	2/400	mg
				Vincristine	7/ 7/70	—	1/2.0	mg
				BCNU	7/ 7/70	—	1/100	g
20 (56, F)	1	Stomach carcinoma: large palpable mass in epigastrium and smaller mass palpable in hypogastrium (19 mos)	1. Surgical treatment 2. Radiotherapy + 5-FU	5-FU	7/ 6/70	7/10/70	5/2500	mg
				NSC-45388	7/ 6/70	7/ 7/70	2/400	mg
				Vincristine	7/ 6/70	—	1/1.5	mg
				BCNU	7/ 6/70	—	1/100	g
21 (65, M)	3	Stomach carcinoma: palpable mass in epigastrium and hepatomegaly (16 mos)	1. Surgical treatment 2. Radiotherapy + 5-FU	5-FU	7/ 6/70 8/17/70	7/10/70 8/21/70	5/2750 5/2500	mg "
							10/5250	"
				NSC-45388	7/ 6/70 7/17/70	7/ 7/70 7/18/70	2/400 2/400	mg "
							4/800	"
				Vincristine	7/ 6/70 8/17/70	— —	1/2.0 1/2.0	mg "
							2/4.0	"
				BCNU	7/ 6/70 8/17/70	— —	1/100 1/100	g "
							2/200	"

Patient data—Continued

Side effects								
	Leukopenia		Thrombocytopenia					
Fall in hgb level (g/100 ml)	Lowest wbc ($\times 10^3$/mm^3)	Duration of (days)	Lowest platelet count ($\times 10^3$/mm^3)	Duration of (days)	Time of maximum hemapoietic depression	Nausea/ vomiting‡	Diar-rhea	Response§: Comment
—	2.3	—	149	—	Day 157	−/−	−	PR (5 mos): Pleural effusion and dyspnea improved considerably after first course of treatment. Performance status improved for 5 months when patient again had pleural effusion and ascites. Autopsy showed metastases of lungs, adrenal glands, and pouch of Douglas.
—	—	—	—	—	—	++/++	−	PD: Patient died on Day 26 because of progressive disease
—	—	—	—	—	—	++/+	−	NE: Patient died on Day 5
0.1	4.9	—	117	—	—	++/+	−	NC

TABLE 1.—

Patient No. (age, sex)	Performance status*	Diagnosis and findings at start of experimental therapy (ET) (duration of disease at start of ET)	Previous treatment†	Combination therapy				
				Drug	From	To	No. of doses/ total dose	
22 (61, F)	1	Breast carcinoma: skin and bone metastases (50 mos)	1. Surgical treatment 2. Radiotherapy 3. CB-3210 4. Cyclophosphamide+ fluoxymesterone 5. Cyclophosphamide+ 6-methylprednisone 6. 5-FU+6-methylprednisone 7. Methotrexate+ 6-methylprednisone 8. Cyclophosphamide+ 6-methylprednisone 9. 5-FU+6-methylprednisone	5-FU	7/ 6/70 8/17/70 10/ 2/70 11/20/70 1/ 8/71 3/12/71	7/ 9/70 8/20/70 10/ 7/70 11/26/70 1/14/71 3/18/71	4/2000 4/2000 4/2000 5/2500 5/2500 5/2500	mg " " " " "
							27/13,500	"
				NSC-45388	7/ 6/70 8/17/70 10/ 2/70 11/20/70 1/ 8/71 3/12/71	7/ 7/70 8/28/70 10/ 5/70 11/21/70 1/ 9/71 3/13/71	2/400 2/400 2/200 2/400 2/200 2/200	mg " " " "
							12/1800	"
				Vincristine	7/ 6/70 8/17/70 10/ 1/70 11/20/70 1/ 8/71 3/12/71	— — — — — —	1/1.7 1/1.7 1/1.7 1/1.7 1/1.7 1/1.5	mg " " " " "
							6/10.0	"
				BCNU	7/ 6/70 8/17/70 10/ 2/70 11/20/70 1/ 8/71 3/12/71	— — — — — —	1/100 1/100 1/100 1/100 1/100 1/100	g " " " " "
						—	6/600	"
23 (61, M)	2	Rectal carcinoma: palpable mass in rectum and hepatomegaly (6 mos)	Radiotherapy+5-FU	5-FU	7/14/70	7/18/70	5/3750	mg
				NSC-45388	7/14/70	7/15/70	2/400	mg
				Vincristine	7/14/70	—	1/1.8	mg
				BCNU	7/14/70	—	1/100	g
24 (49, F)	3	Adenocarcinoma: bone metastases and ascites (22 mos)	1. Surgical treatment 2. Mitomycin C 3. Radiotherapy 4. 5-FU 5. Medroxyprogesterone acetate	5-FU	7/20/70	7/24/70	5/3150	mg
				NSC-45388	7/20/70	7/21/70	2/400	mg
				Vincristine	7/20/70	—	1/1.5	mg
				BCNU	7/20/70	—	1/100	g
25 (54, F)	2	Stomach carcinoma: 10×10 cm mass in right iliac fossa and ascites (3 yrs, 11 mos)	1. Surgical treatment 2. 5-FU+cytosine arabinoside	5-FU	7/27/70	8/10/70	5/3750	mg
				NSC-45388	7/27/70	7/28/70	2/400	mg
				Vincristine	7/27/70	—	1/1.8	mg
				BCNU	7/27/70	—	1/100	g

Patient data—Continued

	Leukopenia		Thrombocytopenia			Side effects		
Fall in hgb level (g/100 ml)	Lowest wbc ($\times 10^3$/mm^3)	Duration of (days)	Lowest platelet count ($\times 10^3$/mm^3)	Duration of (days)	Time of maximum hemapoietic depression	Nausea/ vomiting‡	Diarrhea	Response§: Comment
2.4	2.3	10	127	—	Day 59	+++/+++	++	PR (8 mos): Skin metastases decreased by more than 50% but never cleared completely. No progression of bone metastases.
—	5.5	—	174	—	—	+/−	−	PD: Treatment stopped on Day 37 because an acute abdomen developed and patient was referred for surgical treatment
9.7	5.3	—	150	—	—	+/−	−	PD: Patient died on Day 26
0.6	3.3	7	154	—	—	−/−	−	PD: Patient died on Day 17 as a result of large bowel obstruction

TABLE 1.—

Patient No. (age, sex)	Performance status*	Diagnosis and findings at start of experimental therapy (ET) (duration of disease at start of ET)	Previous treatment†	Combination therapy				
				Drug	From	To	No. of doses/ total dose	
26 (48, M)	1	Colon carcinoma: peritoneal liver and lung metastases ¶¶ (19 mos)	1. Surgical treatment 2. 5-FU+cytosine arabinoside	5-FU	8/ 3/70	8/ 7/70	5/3350	mg
					8/31/70	9/ 4/70	5/3350	"
					10/ 5/70	10/ 9/70	5/3350	"
					11/ 2/70	11/ 6/70	5/3350	"
					11/30/70	12/ 4/70	5/3350	"
					1/ 4/71	1/ 8/71	5/3350	"
					2/ 8/71	2/12/71	5/3350	"
					3/29/71	4/ 2/71	5/3350	"
							40/26,800	"
				NSC-45388	8/ 3/70	8/ 4/70	2/400	mg
					8/31/70	9/ 4/70	2/400	"
					10/ 5/70	10/ 6/70	2/400	"
					11/ 2/70	11/ 3/70	2/400	"
					11/30/70	12/ 1/70	2/400	"
					1/ 4/71	1/ 5/71	2/400	"
					2/ 8/71	2/ 9/71	2/400	"
					3/29/71	3/30/71	2/400	"
							16/3200	"
				Vincristine	8/ 3/70	—	1/1.6	mg
					8/31/70	—	1/1.6	"
					10/ 5/70	—	1/1.6	"
					11/ 2/70	—	1/1.6	"
					11/30/70	—	1/1.6	"
					1/ 4/71	—	1/1.6	"
					2/ 8/71	—	1/1.6	"
					3/29/71	—	1/1.6	"
							8/12.8	"
				BCNU	8/ 3/70	—	1/100	g
					8/31/70	—	1/100	"
					10/ 6/70	—	1/100	"
					11/ 2/70	—	1/100	"
					11/30/70	—	1/100	"
					1/ 4/71	—	1/100	"
					2/ 8/71	—	1/100	"
					3/29/71	—	1/100	"
							8/800	"
27 (43, F)	3	Breast carcinoma: pleural metastases and ovarian infiltration plus skull base infiltration*** (32 mos)	1. Surgical treatment 2. Radiotherapy 3. Oophorectomy 4. Cyclophosphamide+6-methylprednisone 5. Fluoxymesterone+6-methylprednisone+cyclophosphamide	5-FU	7/20/70	7/24/70	5/3000	mg
				NSC-45388	7/20/70	7/21/70	2/400	mg
				Vincristine	7/20/70	—	1/1.5	mg
				BCNU	7/20/70	—	1/100	g

Patient data—Continued

	Side effects							
	Leukopenia		Thrombocytopenia					
Fall in hgb level (g/100 ml)	Lowest wbc ($\times 10^3$/mm^3)	Duration of (days)	Lowest platelet count ($\times 10^3$/mm^3)	Duration of (days)	Time of maximum hemapoietic depression	Nausea/ vomiting‡	Diarrhea	Response§· Comment
0	7.1	—	143	—	—	++/++	–	CR (8 mos): Lung metastases cleared completely, performance status improved considerably; still receiving treatment
1.8	2.4	—	113	—	Day 12	+/+	–	PR: Patient died on Day 45. Autospy showed multiple brain metastases, still leukopenic at death.

TABLE 1.—

Patient No. (age, sex)	Performance status*	Diagnosis and findings at start of experimental therapy (ET) (duration of disease at start of ET)	Previous treatment†	Combination therapy				
				Drug	From	To	No. of doses/ total dose	
28 (27, M)	3	Colon carcinoma: liver metastases plus peritoneal metastases. Infiltration of trigonum of bladder; colectomy. (41 mos)	1. Surgical treatment 2. 5-FU+radiotherapy	5-FU	8/24/70	8/28/70	4/2000	mg
					10/12/70	10/15/70	4/2000	"
					11/30/70	12/ 4/70	5/2500	"
					1/11/71	1/15/71	5/2500	"
					2/22/71	2/25/71	4/2000	"
							22/11,000	"
				NSC-45388	8/24/70	8/25/70	2/400	mg
					10/12/70	10/13/70	2/200	"
					4/30/70	12/ 1/70	2/200	"
					1/11/71	1/12/71	2/200	"
					2/22/71	2/23/71	2/200	"
							10/1200	"
				Vincristine	8/24/70	—	1/1.5	mg
					10/12/70	—	1/1.5	"
					11/30/70	—	1/2.0	"
					1/11/71	—	1/2.0	"
					2/22/71	—	1/2.0	"
							5/9.0	"
				BCNU	8/24/70	—	1/100	g
					10/12/70	—	1/100	"
					11/30/70	—	1/100	"
					1/11/71	—	1/100	"
					2/22/71	—	1/100	"
							5/500	"

Patient data—Continued

	Side effects							
	Leukopenia		Thrombocytopenia					
Fall in hgb level (g/100 ml)	Lowest wbc ($\times 10^3$/mm^3)	Duration of (days)	Lowest platelet count ($\times 10^3$/mm^3)	Duration of (days)	Time of maximum hemapoietic depression	Nausea/ vomiting‡	Diarrhea	Response§: Comment
4.9	4.4	—	146	—	—	++/+	+++	CR: Large abdominal mass cleared. Partial bowel obstruction relieved.

TABLE 1.—

Patient No. (age, sex)	Performance status*	Diagnosis and findings at start of experimental therapy (ET) (duration of disease at start of ET)	Previous treatment†	Combination therapy				
				Drug	From	To	No. of doses/ total dose	
29 (45, M)	2	Colon carcinoma: abdominal metastases (13 yrs)	Surgical treatment	5–FU	8/ 6/70	8/12/70	5/2500	mg
					8/21/70	9/ 4/70	5/2500	"
					10/ 5/70	10/ 9/70	5/2500	"
					11/ 9/70	11/13/70	5/2500	"
					12/14/70	12/18/70	4/2250	"
					1/25/71	1/29/71	5/2500	"
					3/ 1/71	3/ 5/71	5/2500	"
							34/17,250	"
				NSC–45388	8/ 6/70	8/ 7/70	2/400	mg
					8/31/70	9/ 1/70	2/400	"
					10/ 5/70	10/ 6/70	2/400	"
					11/ 9/70	11/10/70	2/400	"
					12/14/70	12/15/70	2/400	"
					1/29/71	1/30/71	2/400	"
					3/ 1/71	3/ 2/71	2/400	"
							14/2800	"
				Vincristine	8/ 6/70	—	1/1.5	mg
					8/31/70	—	1/3.0	"
					10/ 5/70	—	1/2.5	"
					11/ 9/70	—	1/1.5	"
					12/11/70	—	1/1.0	"
					1/29/71	—	1/1.0	"
					3/ 1/71	—	1/1.0	"
							7/11.5	"
				BCNU	8/ 6/70	—	1/100	g
					8/31/70	—	1/100	"
					10/ 5/70	—	1/100	"
					11/ 9/70	—	1/100	"
					12/11/70	—	1/100	"
					1/29/71	—	1/100	"
					3/ 1/71	—	1/100	"
							7/700	

Patient data—Continued

Fall in hgb level (g/100 ml)	Side effects: Leukopenia: Lowest wbc ($\times 10^3$/mm^3)	Side effects: Leukopenia: Duration of (days)	Side effects: Thrombocytopenia: Lowest platelet count ($\times 10^3$/mm^3)	Side effects: Thrombocytopenia: Duration of (days)	Time of maximum hemapoietic depression	Nausea/vomiting‡	Diarrhea	Response§: Comment
3.1	3.9	10	108	—	Day 151	++/+	–	CR (8 mos): Abdominal metastases cleared and performance status improved to normal, weight increased by 8.3 kg. Treatment stopped on Day 248 because of progressive disease.

TABLE 1.—

Patient No. (age, sex)	Performance status*	Diagnosis and findings at start of experimental therapy (ET) (duration of disease at start of ET)	Previous treatment†	Combination therapy				
				Drug	From	To	No. of doses, total dose	
30 (61, F)	2	Colon carcinoma: liver metastases, colostomy ††† (3 mos)	1. Surgical treatment 2. 5-FU+cyclophosphamide+methotrexate+vincristine	5-FU	8/24/70	8/26/70	3/1500	mg
					10/12/70	10/15/70	4/2000	"
					11/16/70	11/19/70	4/2000	"
					1/ 4/71	1/ 6/71	3/1500	"
							14/7000	"
				NSC-45388	8/24/70	8/25/70	2/300	mg
					10/12/70	10/13/70	2/300	"
					11/16/70	11/17/70	2/300	"
					1/ 4/71	1/ 5/71	2/300	"
							8/1200	"
				Vincristine	8/24/70	—	1/1.2	mg
					10/12/70	—	1/1.2	
					11/16/70	—	1/1.2	
					1/ 4/71	—	1/1.2	
							4/4.8	"
				BCNU	8/24/70	—	1/100	g
					10/12/70	—	1/100	"
					11/16/70	—	1/100	"
					1/ 4/71	—	1/100	"
							4/400	"
31 (49, F)	2	Cholangiocellular carcinoma: epigastric mass, hepatomegaly and ascites (3 yrs)	1. Surgical treatment 2. Radiotherapy	5-FU	10/26/70	10/30/70	5/2750	mg
					11/30/70	12/ 4/70	5/2750	"
					1/11/71	1/14/71	4/2000	"
					2/22/71	2/26/71	5/2500	"
							19/10,000	"
				NSC-45388	10/26/70	10/27/70	2/320	mg
					11/30/70	12/ 7/70	2/320	"
					1/11/71	1/12/71	2/300	"
					2/22/71	2/23/71	2/300	"
							8/1240	"
				Vincristine	10/26/70	—	1/1.4	mg
					11/30/70	—	1/1.4	"
					1/11/71	—	1 1.5	"
					2/22/71	—	1/1.5	"
							4/5.8	"
				BCNU	10/26/70	—	1/80	g
					11/30/70	—	1/80	"
					1/11/71	—	1/100	"
					2/22/71	—	1/100	"
							4/360	"

Patient data—Continued

Fall in hgb level (g/100 ml)	Side effects: Leukopenia: Lowest wbc ($\times 10^3$/mm^3)	Leukopenia: Duration of (days)	Thrombocytopenia: Lowest platelet count ($\times 10^3$/mm^3)	Thrombocytopenia: Duration of (days)	Time of maximum hemapoietic depression	Nausea/vomiting‡	Diarrhea	Response§: Comment
1.1	6.9	—	200	—	—	+/−	+++	PR (4 mos): Dosage reduced because of severe diarrhea. Died on Day 160 because of progressive disease.
0.9	2.6	5	210	—	Day 18	++/++	−	PR (4 mos): Epigastric mass and hepatomegaly decreased in size. Ascites controlled.

TABLE 1.—

Patient No. (age, sex)	Performance status*	Diagnosis and findings at start of experimental therapy (ET) (duration of disease at start of ET)	Previous treatment†	Combination therapy				
				Drug	From	To	No. of doses/ total dose	
32 (60, M)	1	Rectal carcinoma: liver metastases (15 mos)	Surgical treatment	5–FU	10/28/70	11/ 1/70	5/3150	mg
					11/30/70	12/ 4/70	5/3150	"
							10/6300	"
				NSC–45388	10/28/70	10/29/70	2/400	mg
					11/30/70	12/ 1/70	2/400	"
							4/800	"
				Vincristine	10/28/70	—	1/1.5	mg
					11/30/70	—	1/1.5	"
							2/3.0	"
				BCNU	10/28/70	—	1/100	g
					11/30/70	—	1/100	"
							2/200	"
33 (80, M)	1	Colon carcinoma: lung metastases (multiple) and paraaortic glands (11 mos)	1. Surgical treatment 2. Radiotherapy+5–FU	5–FU	11/ 2/70	11/ 6/70	5/3750	mg
					12/14/70	12/18/70	5/3750	
					1/25/71	1/29/71	5/3750	
					3/ 1/71	3/ 5/71	5/3750	
							20/15,000	"
				NSC–45388	11/ 2/70	11/ 3/70	2/400	mg
					12/14/70	12/15/70	2/400	"
					1/25/71	1/26/71	2/400	"
					3/ 1/71	3/ 2/71	2/400	"
							8/1600	"
				Vincristine	11/ 2/70	—	1/2.0	mg
					12/14/70	—	1/2.0	"
					1/25/71	—	1/2.0	"
					3/ 1/71	—	1/2.0	"
							4/8.0	"
				BCNU	11/ 2/70	—	1/100	g
					12/14/70	—	1/100	"
					1/25/71	—	1/100	"
					3/ 1/71	—	1/100	"
							4/400	"

Patient data—Continued

Side effects								
	Leukopenia		Thrombocytopenia					
Fall in hgb level (g/100 ml)	Lowest wbc ($\times 10^3$/mm^3)	Duration of (days)	Lowest platelet count ($\times 10^3$/mm^3)	Duration of (days)	Time of maximum hemapoietic depression	Nausea/ vomiting‡	Diarrhea	Response§: Comment
2.5	8.6	—	274	—	—	+/+	–	PD: Patient died on Day 40. Autopsy showed multiple liver metastases and microscopic lung metastases.
1.2	4.5	—	155	—	—	+/–	–	CR (4 mos): Lung metastases cleared. Patient died suddenly on Day 131.

TABLE 1.—

Patient No. (age, sex)	Performance status*	Diagnosis and findings at start of experimental therapy (ET) (duration of disease at start of ET)	Previous treatment†	Combination therapy				
				Drug	From	To	No. of doses/ total dose	
34 (48, F)	1	Ovarian carcinoma: pelvic and peritoneum metastases (9 mos)	1. Surgical treatment 2. Chlorambucil	5–FU	11/ 2/70 12/ 7/70 1/11/71 3/ 1/71	11/ 6/70 12/11/70 1/15/71 3/ 5/71	5/3750 5/3750 5/3750 5/3750	mg " " "
							20/15,000	"
				NSC–45388	11/ 2/70 12/ 7/70 1/11/71 3/ 1/71	11/ 3/70 12/ 8/70 1/12/71 3/ 2/71	2/400 2/400 2/400 2/400	mg " " "
							8/1600	"
				Vincristine	11/ 2/70 12/ 7/70 1/11/71 3/ 1/71	— — — —	1/2.0 1/2.0 1/2.0 1/2.0	mg " " "
							4/8.0	"
				BCNU	11/ 2/70 12/ 7/70 1/11/71 3/ 1/71	— — — —	1/100 1/100 1/100 1/100	g " " "
							4/400	"
35 (62, M)	2	Colon carcinoma: mesenteric pancreatic and small bowel infiltration (15 mos)	Surgical treatment	5–FU	11/ 2/70 12/ 7/70 1/11/71	11/ 6/70 12/10/70 1/15/71	5/4100 4/3280 5/3750	mg " "
							14/11,130	"
				NSC–45388	11/ 2/70 12/ 7/70 1/11/71	11/ 3/70 12/ 8/80 1/12/71	2/400 2/400 2/400	" " "
							6/1200	"
				Vincristine	11/ 2/70 12/ 7/70 1/11/71	— — —	1/2.0 1/2.0 1/2.0	" " "
							3/6.0	"
				BCNU	11/ 2/70 12/ 7/70 1/11/71	— — —	1/100 1/100 1/100	g " "
							3/300	

Patient data—Continued

Fall in hgb level (g/100 ml)	Leukopenia: Lowest wbc ($\times 10^3$/mm^3)	Leukopenia: Duration of (days)	Thrombocytopenia: Lowest platelet count ($\times 10^3$/mm^3)	Thrombocytopenia: Duration of (days)	Time of maximum hemapoietic depression	Nausea/vomiting‡	Diarrhea‡	Response§: Comment
3.7	1.7	70	115	—	Day 139	+++/+++	–	PR (3 mos): Pelvic metastases decreased in size and performance status improved.
3.3	6.5	—	126	—	—	+/+	+	NC: Disease remained static for 3 months. Treatment stopped because of progressive disease on Day 106.

TABLE 1.—

Patient No. (age, sex)	Performance status*	Diagnosis and findings at start of experimental therapy (ET) (duration of disease at start of ET)	Previous treatment†	Combination therapy				
				Drug	From	To	No. of doses/ total dose	
36 (46, M)	2	Stomach carcinoma: multiple lung metastases (6 mos)	1. Surgical treatment 2. Radiotherapy+5-FU	5-FU	11/23/70 1/11/71 2/15/71 3/29/71	11/27/70 1/14/71 2/19/71 4/ 2/71	5/3000 5/3000 5/3000 5/3000	mg " " "
							20/12,000	"
				NSC-45388	11/23/70 1/11/71 2/15/71 3/29/71	11/24/70 1/12/71 2/16/71 3/30/71	2/400 2/400 2/400 2/400	mg " " "
							8/1600	"
				Vincristine	11/23/70 1/11/71 2/15/71 3/29/71	— — — —	1/2.0 1/2.0 1/2.0 1/1.5	mg " " "
							4/7.5	"
				BCNU	11/ 3/70 1/11/71 2/15/71 3/29/71	— — — —	1/100 1/100 1/100 1/100	g " " "
							4/400	"
37 (66, F)	1	Adenocarcinoma, origin unknown: liver metastases (1 mo)	—	5-FU	12/14/70	12/18/70	4/3000	mg
				NSC-45388	12/14/70	12/15/70	2/400	mg
				Vincristine	12/14/70	—	1/2.0	mg
				BCNU	12/14/70	—	1/100	g
38 (44, F)	1	Colon carcinoma: diffuse abdominal and pelvic metastases (28 mos)	1. Surgical treatment 2. 5-FU+cytosine arabinoside 3. 5-FU 4. Radiotherapy+5-FU+vincristine	5-FU	12/14/70	12/18/70	4/2000	mg
				NSC-45388	12/14/70	12/15/70	2/400	mg
				Vincristine	12/14/70	—	1/2.0	mg
				BCNU	12/14/70	—	1/100	g

Patient data—Continued

Fall in hgb level (g/100 ml)	Leukopenia: Lowest wbc ($\times 10^3$/mm^3)	Leukopenia: Duration of (days)	Thrombocytopenia: Lowest platelet count ($\times 10^3$/mm^3)	Thrombocytopenia: Duration of (days)	Time of maximum hemapoietic depression	Nausea/vomiting‡	Diarrhea‡	Response§: Comment
1.6	2.5	7	173	—	Day 22	++/++	–	Imp: Performance status improved for 2 months. Lung metastases increased in size. Patient died on Day 137.
1.6	3.3	10	171	—	Day 22	+/–	–	PD: Massive hepatomegaly and ascites developed. Treatment stopped because of progressive disease.
—	3.1	—	132	—	—	+/–	–	PD: Treatment stopped on Day 36 because of progressive disease.

TABLE 1.—

Patient No. (age, sex)	Performance status*	Diagnosis and findings at start of experimental therapy (ET) (duration of disease at start of ET)	Previous treatment†	Combination therapy				
				Drug	From	To	No. of doses/ total dose	
39 (48, F)	1	Adenocarcinoma, origin unknown: mesenteric and small bowel metastases (24 mos)	Surgical treatment	5-FU	1/11/71	1/15/71	5/2500	mg
					2/15/71	2/19/71	5/2500	"
					3/15/71	3/14/71	5/2500	"
							15/7500	"
				NSC-45388	1/11/71	1/12/71	2/400	mg
					2/15/71	2/16/71	2/400	"
					3/15/71	3/16/71	2/400	"
							6/1200	"
				Vincristine	1/11/71	—	1/1.2	mg
					2/15/71	—	1/1.2	"
					3/15/71	—	1/1.2	"
							3/3.6	"
				BCNU	1/11/71	—	1/100	g
					2/15/71	—	1/100	"
					3/15/71	—	1/100	"
							3/300	"
40 (64, M)	3	Cecum carcinoma: pulmonary metastases, liver metastases with jaundice ‡‡‡ (12 mos)	Surgical treatment	5-FU	1/11/71	1/15/71	5/3750	mg
					2/ 1/71	2/ 5/71	5/3750	"
					3/ 8/71	3/12/71	5/3750	"
							15/11,250	"
				NSC-45388	1/11/71	1/12/71	2/400	mg
					2/ 1/71	2/ 2/71	2/400	"
					3/ 8/71	3/ 9/71	2/400	"
							6/1200	"
				Vincristine	1/11/71	—	1/2.0	mg
					2/ 1/71	—	1/2.0	"
					3/ 8/71	—	1/2.0	"
							3/6.0	"
				BCNU	1/11/71	—	1/100	g
					2/ 1/71	—	1/100	"
					3/ 8/71	—	1/100	"
							3/300	"
41 (66, M)	2	Carcinoid: liver metastases (28 mos)	5-FU + cytosine arabinoside	5-FU	1/13/71	1/19/71	5/2500	mg
				NSC-45388	1/13/71	1/14/71	2/400	mg
				Vincristine	1/13/71	—	1/1.4	mg
				BCNU	1/13/71	—	1/100	g

Patient data—Continued

Side effects								
	Leukopenia		Thrombocytopenia					
Fall in hgb level (g/100 ml)	Lowest wbc ($\times 10^3$/mm^3)	Duration of (days)	Lowest platelet count ($\times 10^3$/mm^3)	Duration of (days)	Time of maximum hemapoietic depression	Nausea/ vomiting‡	Diar-rhea‡	Response§: Comment
1.1	5.0	—	106	—	—	++/++	–	NC: Disease remains static
0.5	5.0	—	74	10	Day 5	+/–	–	PR (2 mos): Hepatomegaly decreased by 90% and jaundice cleared. Pulmonary metastases decreased in size. Performance status improved considerably.
—	1.9	—	188	—	Day 5	+/+	–	PD: Small bowel obstruction developed on Day 12. Patient died after surgical treatment. Laparotomy showed small primary of terminal ileum and liver metastases.

TABLE 1.—

Patient No. (age, sex)	Performance status*	Diagnosis and findings at start of experimental therapy (ET) (duration of disease at start of ET)	Previous treatment†	Combination therapy				
				Drug	From	To	No. of doses/ total dose	
42 (66, M)	3	Stomach carcinoma: adbominal metastases (24 mos)	Surgical treatment	5–FU	11/26/70	12/ 2/70	5/2100	mg
					1/18/71	1/22/71	5/2250	"
							10/4350	"
				NSC–45388	11/26/70	11/27/70	2/240	mg
					1/18/71	1/19/71	2/240	"
							4/480	"
				Vincristine	11/26/70	—	1/1.0	mg
					1/18/71	—	1/1.0	"
							2/2.0	"
				BCNU	11/ 6/70	—	1/70	g
					1/18/71	—	1/70	"
							2/140	"
43 (47, F)	2	Adenocarcinoma, origin unknown: liver and pulmonary metastases (2 mos)	—	5–FU	1/25/71	1/29/71	5/2500	mg
					2/22/71	2/26/71	5/3250	"
							10/5750	"
				NSC–45388	1/25/71	1/26/71	2/400	mg
					2/22/71	2/23/71	2/400	"
							4/800	"
				Vincristine	1/25/71	—	1/2.0	mg
					2/22/71	—	1/2.0	"
							2/4.0	"
				BCNU	1/25/71	—	1/100	g
					2/22/71	—	1/100	"
							2/200	"

Patient data—Continued

	Side effects							
	Leukopenia		Thrombocytopenia					
Fall in hgb level (g/100 ml)	Lowest wbc ($\times 10^3$/mm^3)	Duration of (days)	Lowest platelet count ($\times 10^3$/mm^3)	Duration of (days)	Time of maximum hemapoietic depression	Nausea/ vomiting‡	Diarrhea‡	Response§: Comment
4.1	5.1	—	30	10	Day 40	+/−	−	Imp: Performance status improved with weight gain of 17 kg. Abdominal metastases became more discrete. Died suddenly at home on Day 81.
3.5	4.4	—	150	—	—	+/+	−	NC: Treatment stopped on Day 64.

TABLE 1.—

Patient No. (age, sex)	Performance status*	Diagnosis and findings at start of experimental therapy (ET) (duration of disease at start of ET)	Previous treatment†	Combination therapy				
				Drug	From	To	No. of doses/total dose	
44 (46, M)	2	Rectal carcinoma: liver metastases (18 mos)	Surgical treatment	5-FU	11/18/70	11/24/70	5/3250	mg
					12/28/70	12/31/70	4/3000	"
					2/ 2/71	2/ 5/71	5/3750	"
					3/22/71	3/24/71	2/1500	"
							16/11,500	"
				NSC-45388	11/18/70	11/19/70	2/400	mg
					12/28/70	12/29/70	2/400	"
					2/ 2/71	2/ 3/71	2/400	"
					3/22/71	3/24/71	2/400	"
							8/1600	"
				Vincristine	11/18/70	—	1/2.5	mg
					12/28/70	—	1/2.5	"
					2/ 2/71	—	1/2.5	"
					3/22/71	—	1/2.5	"
							4/10	"
				BCNU	11/18/70	—	1/200	g
					12/28/70	—	1/200	"
					2/ 2/71	—	1/200	"
					3/22/71	—	1/200	"
							4/800	"
45 (52, M)	1	Rectal carcinoma: abdominal and pelvic metastases (29 mos)	1. Surgical treatment 2. Radiotherapy + 5-FU	5-FU	2/ 1/71	2/ 5/71	5/3750	mg
					3/15/71	3/19/71	5/3750	"
							10/7500	"
				NSC-45388	2/ 1/71	2/ 2/71	2/400	mg
					3/15/71	3/16/71	2/400	"
							4/800	"
				Vincristine	2/ 1/71	—	1/2.0	mg
					3/15/71	—	1/2.0	"
							2/4.0	"
				BCNU	2/ 1/71	—	1/100	g
					3/15/71	—	1/100	"
							2/200	"

Patient data—Continued

Side effects								
	Leukopenia		Thrombocytopenia					
Fall in hgb level (g/100 ml)	Lowest wbc ($\times 10^3$/mm^3)	Duration of (days)	Lowest platelet count ($\times 10^3$/mm^3)	Duration of (days)	Time of maximum hemapoietic depression	Nausea/ vomiting‡	Diar-rhea‡	Response§: Comment
4.6	7.3	—	58	21	Day 62	+/+	+++	PR (2 mos): Performance status improved and hepatomegaly decreased in size by 60%. Fourth course of treatment interrupted because of severe diarrhea. Still receiving treatment.
—	5.6	—	155	—	—	+/++	−	NC

TABLE 1.—

Patient No. (age, sex)	Performance status*	Diagnosis and findings at start of experimental therapy (ET) (duration of disease at start of ET)	Previous treatment†	Combination therapy				
				Drug	From	To	No. of doses/ total dose	
46 (47, F)	3	Breast carcinoma: skin metastases and brain metastases (21 mos)	1. Surgical treatment 2. Radiotherapy 3. Oophorectomy 4. Vincristine+5-FU+ methotrexate+ cyclophosphamide+ 6-methylprednisone 5. Cyclophosphamide+ fluoxymesterone 6. Mitomycin C 7. Butyraldehyde, 3-hydroxy-2-oxo-, bis (thiosemicarbazone), butyrate (ester) (60-C-63)	5-FU	2/ 8/71	2/12/71	5/3100	mg
				NSC-45388	2/ 8/71	2/ 9/71	2/400	mg
				Vincristine	2/ 8/71	—	1/1.5	m
				BCNU	2/ 8/71	—	1/100	g

*1 = Mild; 2 = moderate; 3 = severe; 4 = life threatening.
†See List of Compounds at end of text for NSC numbers and chemical nomenclature of compounds.
‡++++ = Life threatening or contributing cause of death; +++ = severe; ++ = moderate; + = mild; − = none.
§CR = complete remission; PR = partial remission; Imp = improved; NC = no change; PD = progressive disease; NE = not evaluable.
¶Measurable lung lesions decreased by more than 50% for 2 months while the osteolytic lesions in the pelvis and femur showed signs of recalcification.
**All measurable lesions decreased by more than 50%. Small lesions cleared completely and the ulcerations healed.
††Pulmonary lesions cleared for 3 months. Osteolytic lesions became osteosclerotic.
‡‡Patient improved for at least 4 months but was considered PR for 1 month only.

Patient data—Continued

	Side effects							
	Leukopenia		Thrombocytopenia					
Fall in hgb level (g/100 ml)	Lowest wbc ($\times 10^3$/mm^3)	Duration of (days)	Lowest platelet count ($\times 10^3$/mm^3)	Duration of (days)	Time of maximum hemapoietic depression	Nausea/ vomiting‡	Diarrhea‡	Response§: Comment
—	3.0	2+	55	2+	Day 11	+/−	−	PD: Died on Day 13. Autopsy showed ulcerative enterocolitis and intraarterial lung tumor emboli.

§§The pleural effusion cleared without therapeutic thoracocentesis.

¶¶Followup, September 1971: patient still in CR after 1 year and continues to receive courses of treatment.

***Pleural effusion cleared with thoracocentesis, and signs of skull base infiltration improved for 28 days. Patient became confused on Day 37 and comatose on Day 40.

†††Liver metastases decreased in size by more than 50% as measured clinically and by isotope uptake. Serum albumin rose and serum alkaline phosphatase fell.

‡‡‡Lung metastases decreased by 80% (and have subsequently cleared completely). Hepatomegaly cleared clinically. Liver scan showed 90% decrease in size of measurable metastases. Serum bilirubin decreased from 8.8 mg/100 ml to less than 1 mg/100 ml.

MATERIALS AND METHODS

Forty-six patients with advanced inoperable cancer were treated with this combination of drugs. The dose schedules were as follows: 5-FU, 10 mg/kg/day intravenously (iv) on Days 1–5; NSC-45388, 3 mg/kg/day iv on Days 1 and 2; vincristine, 0.025 mg/kg iv on Day 1; and BCNU, 1.5 mg/kg iv on Day 1. The agents were injected via running iv infusion. If toxic effects occurred without therapeutic response, the patient was considered to have had an adequate trial. If therapeutic response was noted, subsequent courses of treatment were given when hemapoietic toxicity had cleared or at monthly intervals.

At the start of treatment the cases were evaluated for performance status, and measurable disease was recorded clinically and radiologically. A full blood count was performed at the start of treatment and at regular intervals thereafter. Tests for serum electrophoretic pattern, alkaline phosphatase, bilirubin, serum glutamic oxaloacetic transaminase, urea, uric acid, and creatinine were done at the start of treatment and at 2-week intervals thereafter.

Of the 46 patients treated the cases of 43 were evaluable, 12 with breast cancer, 21 with gastrointestinal carcinoma, five with adenocarcinoma of unknown origin, three with ovarian carcinoma, one with uterine carcinoma, and one with myeloma. To be evaluable the patient had to have received enough drug and have lived long enough to show measurable side effects or clinical response or both. The data for each patient at the start of treatment, including the previous treatment and the exact dose of each drug given, are shown in table 1.

RESULTS

Toxic Effects

Nausea was the most common side effect, occurring in 41 of 46 patients treated. It was most severe on Day 1 and was accompanied by vomiting in 21 patients. Nausea seldom persisted for more than 48 hours. Diarrhea occurred in nine patients but was severe in only three. A fall in the hemoglobin level of more than 2 g/100 ml occurred in 24 patients. A white blood cell count less than 4000/mm^3 was noted in 20 patients and a platelet count of less than 100,000/mm^3 was observed in 11 patients. Alopecia was common and was pronounced in all patients receiving more than one course of treatment. Venous spasm and arm pain occurred commonly during the administration of NSC-45388 and BCNU.

The side effects are shown in table 2. The time of maximum hemapoietic depression is shown in table 1.

Therapeutic Effects

Complete remission was defined as disappearance of all measurable lesions for at least 1 month. Partial remission was defined as a decrease in the size of all measurable lesions of at least 50%, lasting for at least 1 month, without the appearance of new lesions. Improvement was defined as a decrease of less than 50% of all measurable lesions, lasting for at least 1 month. In patients having complete remission,

TABLE 2.—Toxic effects

Toxic effect				Number of patients
Fall in hemoglobin level >2 g/100 ml				24/43
Leukopenia (<4000 cells/mm^3)				20/43
Thrombocytopenia (<100,000 cells/mm^3)				11/43
Alopecia				All patients receiving more than 1 course of treatment
	Mild	*Moderate*	*Severe*	*Total*
Nausea	27	12	2	41/46
Vomiting	12	7	2	21/46
Diarrhea	2	4	3	9/46

TABLE 3.—Response to treatment*

Tumor type	No. of patients	CR	PR	Imp	NC	PD	NE
Breast	12		6	1	1	4	
Gastrointestinal (23)							
Stomach	5			2	1	1	1
Colon	8	4	2		1	1	
Rectum	7		1	1	1	3	1
Carcinoid	1					1	
Cholangiocellular	2		2				
Adenocarcinoma, primary unknown	6		1		2	2	1
Ovary	3		2	1			
Uterus	1					1	
Myeloma	1		1				
Totals	46	4	15	5	6	13	3

*CR = complete remission; PR = partial remission; Imp = improved; NC = no change; PD = progressive disease; NE = not evaluable.

TABLE 4.—Response to treatment in 34 previously treated patients

Tumor type	No. of patients previously treated/total No. of patients	CR (No. of patients previously treated/total No. of patients)	PR	Imp	NC	PD
Breast	12/12		6/6	1/1	1/1	4/4
Gastrointestinal	15/21	3/4	3/5	2/3	2/3	5/6
Adenocarcinoma, primary unknown	2/5		1/1		0/2	1/2
Ovary	3/3		2/2	1/1		
Uterus	1/1					1/1
Myeloma	1/1		1/1			
Totals	34/43	3/4	13/5	4/5	3/6	11/13

partial remission, or improvement, subjective benefit was also a prerequisite.

Seven of 12 patients with breast cancer, two of five patients with stomach carcinoma, six of eight patients with colon carcinoma, two of seven patients with rectal carcinoma, two of two patients with cholangiocellular carcinoma, one of six patients with adenocarcinoma of unknown origin, three of three patients with ovarian carcinoma, and one patient with multiple myelomatosis responded to treatment.

Complete remission occurred in four of eight patients with colon carcinoma. Partial remission occurred in six patients with breast cancer, five patients with gastrointestinal carcinoma, and two patients with ovarian carcinoma. These results are shown in table 3.

Thirty-four of the 43 patients with evaluable cases had received prior chemotherapy as shown in table 1. Prior chemotherapy in no way precluded response to the 4-drug combination. Of the 11 patients with gastrointestinal carcinoma who responded to treatment, eight had had prior chemotherapy as had the seven patients with breast cancer and the three patients with ovarian carcinoma who responded. These results are shown in table 4.

At the present time two of the patients with colon carcinoma (Nos. 26 and 28) who had complete remissions are still in remission and con-

tinue to receive courses of treatment. Four of the patients with partial response (No. 31 with cholangiocellular carcinoma, No. 34 with ovarian carcinoma, No. 40 with colon carcinoma, and No. 44 with rectal carcinoma) continue to receive courses of treatment. One patient (No. 39 with adenocarcinoma of unknown origin), although evaluated as "no change," continues to receive courses of the 4-drug combination. All patients who responded with complete remission, partial remission, or improvement had total benefit from treatment in that the therapeutic response outweighed the toxic effects.

DISCUSSION AND CONCLUSIONS

5-FU and vincristine are known to be effective in gastrointestinal cancer and breast cancer and a clinical trial with NSC-45388 in gastrointestinal cancer has been carried out by Moertel et al (12). BCNU is known to be of some value in gastrointestinal cancer. It was decided to combine the four agents in such a way as to decrease toxic effects and yet increase therapeutic response.

Worthwhile objective response (complete remission plus partial remission) was noted in 19 of 43 patients treated with this combination therapy, while five patients improved to a lesser degree (improvement). Of particular significance are the good responses noted in six of eight patients with advanced colon carcinoma. The rate and quality of response observed was greater than that expected for any of the agents used alone. The response in patients with gastrointestinal carcinoma, ovarian carcinoma, and breast cancer was encouraging. The side effects encountered were tolerable. In relation to the therapeutic effect the side effects were considered minor. This is in contrast to previous studies of combination therapy in gastrointestinal tract carcinoma where uniformly bad results have been obtained (13,14).[5]

Further clinical trials of this 4-drug combination are indicated.

LIST OF COMPOUNDS

CB-3210: NSC-37023; serine, ester with bis(2-chloropropyl)carbamic acid, DL-
Chlorambucil: NSC-3088; butyric acid, 4-[*p*-[bis(2-chloroethyl)amino]phenyl]-
Cyclophosphamide: NSC-26271; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate
Cytosine arabinoside: NSC-63878; cytosine, 1-β-D-arabinofuranosyl-, monohydrochloride
Diethylstilbestrol: NSC-3070; 4,4'-stilbenediol, α,α'-diethyl-
Dromostanolone propionate: NSC-12198; 5α-androstan-3-one, 17β-hydroxy-2α-methyl-, propionate
Fluoxymesterone: NSC-12165; androst-4-en-3-one, 9-fluoro-11β, 17β-dihydroxy-17-methyl-
Medroxyprogesterone acetate: NSC-26386; pregn-4-ene-3,20-dione, 17-hydroxy-6α-methyl-, acetate
Methotrexate: NSC-740; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-
6-α-Methylprednisolone: NSC-19987; pregna-1,4-diene-3,20-dione, 11β,17,21-trihydroxy-6α-methyl-
Mitomycin C: NSC-26980
Phenylalanine mustard: NSC-14210; alanine, 3-[*p*-[bis(2-chloroethyl)amino]phenyl]-, monohydrochloride, DL-
L-Phenylalanine mustard: NSC-8806; alanine, 3-[*p*-[bis(2-chloroethyl)amino]phenyl]-, monohydrochloride, L-

[5] Gailani, S. Results of treatment of gastrointestinal cancer with fluorouracil and cytosine arabinoside. Unpublished data, Acute Leukemia Group B, 1971.

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ANNOUNCEMENTS

Placement Service for Clinical Oncologists

The American Society of Clinical Oncology has initiated a placement service for clinical oncologists—the Clearinghouse of Oncologic Opportunities. It will accept inquiries from applicants concerning staff positions and oncology fellowships, as well as announcements of openings for such applicants. The applicant names will be kept completely confidential upon request. The service is open to members and nonmembers of the ASCO.

Please direct inquiries and announcements to Mary E. Sears, MD, American Society of Clinical Oncology, University of Rochester Medical Center, 1351 Mt. Hope Ave, Rochester, NY 14620.

Cancer Fellowships

Postgraduate Fellowships in cancer for practicing physicians are available at the University of Southern California School of Medicine and the Los Angeles County-University of Southern California Medical Center. These Fellowships are sponsored by the National Cancer Institute, are of one month's duration, and carry a stipend of $750. Separate programs are available in gynecologic oncology, medical oncology, radiation therapy, tumor pathology, and tumor surgery. Applicants should be Board eligible or Board certified and should submit a résumé of their professional background to Arthur J. Donovan, MD, Program Director, Cancer Training Program, School of Medicine, University of Southern California, 2025 Zonal Avenue, Los Angeles, Calif 90033.

Current Concepts in the Treatment of Malignant Disease

On May 18 and 19, 1972, the Chemotherapy Program of the National Cancer Institute is sponsoring a course on Current Concepts in the Treatment of Malignant Disease. The course will be geared to the general physician with an interest in oncology problems. It will attempt to review the current therapeutic approaches to many of the commonly encountered malignant tumors. In addition, an attempt will be made to highlight some of the new advances in cancer therapy, such as new drugs, immunologic approaches to cancer therapy, and the implications of pharmacology in the use of antineoplastic drugs. Specific areas to be covered include the therapy of gastrointestinal cancer, breast cancer, lung cancer, ovarian cancer, lymphomas, and testicular tumors. The organizers of the course are Dr. Paul P. Carbone, Dr. Stephen K. Carter, and Dr. G. Lennard Gold. The course is approved for credit by The Academy of General Practice. Interested physicians can obtain further information by writing to the Scientific Director for Chemotherapy, National Cancer Institute, Bethesda, Maryland 20014.

ANNOUNCEMENTS

XIth International Cancer Congress

The Eleventh International Cancer Congress organized under the auspices of the UICC will be centered in Florence, Italy (October 20–26, 1974). Following the opening ceremony at the Palazzo Vecchio, the first 2 days will be devoted to special sessions organized mainly on a unidisciplinary basis, in Florence and in nearby towns (Montecatini, Lucca, Siena, and Perugia). During the following days ordinary sessions of the Congress will be held in Florence itself. The programme comprises multidisciplinary symposia in the mornings and workshops or presentations of proffered papers in the afternoons. A memorial lecture will be delivered at the closing ceremony. Advanced courses in clinical oncology are scheduled to follow the Congress. The projection of scientific films and the display of scientific and commercial exhibits are planned. Forms for registration and hotel accommodation as well as information on travel, tours, and special events will be provided in the 2nd Circular to be issued in October 1972. Families and guests accompanying members must be registered as associate members. English, French, and Italian will be the official languages of the Congress with simultaneous interpretation at most sessions.

Dates

Opening ceremony: October 20 (Sunday afternoon)
Special sessions: October 21 and 22 (Monday and Tuesday)
Ordinary sessions: October 23–26 (Wednesday to Saturday)
Closing ceremony: October 26 (Saturday)
Advanced courses: October 28 and 29 (Monday and Tuesday)

For information write to:

XI INTERNATIONAL CANCER CONGRESS
Istituto Nazionale per lo Studio e la Cura dei Tumori
20133 Milano, Via G. Venezian 1, Italy

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